

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification⁴ : C12N 15/00, C12P 21/02 A61K 39/395, 49/00	A1	(11) International Publication Number: WO 89/06690 (43) International Publication Date: 27 July 1989 (27.07.89)
(21) International Application Number: PCT/US89/00238 (22) International Filing Date: 23 January 1989 (23.01.89) (31) Priority Application Number: 147,351 (32) Priority Date: 22 January 1988 (22.01.88) (33) Priority Country: US (71) Applicant: THE GENERAL HOSPITAL CORPORATION [US/US]; Fruit Street, Boston, MA 02114 (US). (72) Inventor: SEED, Brian ; 47A Joy Street, Boston, MA 02114 (US). (74) Agents: GOLDSTEIN, Jorge, A. et al.; Saidman, Sterne, Kessler & Goldstein, 1225 Connecticut Avenue, N.W., Suite 300, Washington, DC 20036 (US).	(81) Designated States: AU, DK, FI, JP, KR. Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	

(54) Title: CLONED GENES ENCODING IG-CD4 FUSION PROTEINS AND THE USE THEREOF

(57) Abstract

The invention relates to a fusion protein which comprises an immunoglobulin of the IgM, IgG1 or IgG3 immunoglobulin class, wherein the variable region of the light or heavy chain has been replaced with CD4 or fragment thereof which is capable of binding to gp120. The invention also relates to an immunoglobulin-like molecule comprising the fusion protein of the invention together with an immunoglobulin light or heavy chain. The invention also relates to a method of treating HIV or SIV infection comprising administering the fusion proteins or immunoglobulin-like molecules of the invention to an animal. The invention also relates to assays for HIV or SIV comprising contacting a sample suspected of containing HIV or SIV gp120 with the immunoglobulin-like molecule of fusion protein of the invention, and detecting whether a complex is formed.

Applicants: Gary Beaudry and
Paul J. Maddon
U.S. Serial No. 08/485,163
Filed: June 7, 1995
Exhibit 6

-1-

TITLE OF THE INVENTION

CLONED GENES ENCODING IG-CD4 FUSION PROTEINS AND THE USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of U.S. Application Serial No. 07/147,351 filed January 22, 1988.

FIELD OF THE INVENTION

The invention is in the field of recombinant genetics.

BACKGROUND OF THE INVENTION

The human and simian immunodeficiency viruses HIV and SIV are the causative agents of Acquired Immune Deficiency Syndrome (AIDS) and Simian Immunodeficiency Syndrome (SIDS), respectively. See Curren, J. *et al.*, *Science* 329:1359-1357 (1985); Weiss, R. *et al.*, *Nature* 324:572-575 (1986). The HIV virus contains an envelope glycoprotein, gp120 which binds to the CD4 protein present on the surface of helper T lymphocytes, macrophages and other cells. Dalglish *et al.*, *Nature*, 312:763 (1984). After the gp120 binds to CD4, virus entry is facilitated by an envelope-mediated fusion of the viral target cell membranes.

During the course of infection, the host organism develops antibodies against viral proteins, including the major envelope glycoproteins gp120 and gp41. Despite this humoral immunity, the disease progresses, resulting in a lethal immunosuppression characterized by multiple opportunistic infections, parasitemia, dementia and death. The failure of host anti-viral antibodies to arrest the progression of the disease represents one of the most vexing and alarming aspects of the infection, and augurs poorly for vaccination efforts based upon conventional approaches.

Two factors may play a role in the inefficacy of the humoral response to immunodeficiency viruses. First, like other RNA viruses (and like retroviruses in particular), the immunodeficiency viruses show a high mutation rate which allows antigenic variation to progress at a high rate in response to host immune surveillance. Second, the envelope glycoproteins themselves are heavily glycosylated molecules presenting few epitopes suitable for high affinity antibody binding. The poorly antigenic, "moving" target which the viral envelope presents, allows the host little opportunity for restricting viral infection by specific antibody production.

Cells infected by the HIV virus express the gp120 glycoprotein on their surface. Gp120 mediates fusion events among CD4⁺ cells via a reaction similar to that by which the virus enters the uninfected cell, leading to the formation of short-lived multinucleated giant cells. Syncytium formation is dependent on a direct interaction of the gp120 envelope glycoprotein with the CD4 protein. Dalgleish *et al.*, *supra*, Klatzmann, D. *et al.*, *Nature* 312:763 (1984); McDougal, J.S. *et al.*, *Science*, 231:382 (1986); Sodroski, J. *et al.*, *Nature*, 322:470 (1986); Lifson, J.D. *et al.*, *Nature*, 323:725 (1986); Sodroski, J. *et al.*, *Nature*, 321:412 (1986).

The CD4 protein consists of a 370 amino acid extracellular region containing four immunoglobulin-like domains, a membrane spanning domain, and a charged intracellular region of 40 amino acid residues.

Maddon, P. *et al.*, Cell 42:93 (1985); Clark, S. *et al.*, Proc. Natl. Acad. Sci. (USA) 84:1649 (1987).

Evidence that CD4-gp120 binding is responsible for viral infection of cells bearing the CD4 antigen includes the finding that a specific complex is formed between gp120 and CD4. McDougal *et al.*, *supra*. Other workers have shown that cell lines, which were non-infective for HIV, were converted to infectable cell lines following transfection and expression of the human CD4 cDNA gene. Maddon *et al.*, Cell 47:333-348 (1986).

In contrast to the majority of antibody-envelope interactions, the receptor-envelope interaction is characterized by a high affinity ($K_a = 10^8$ /mole) immutable association. Moreover, the affinity of the virus for CD4 is at least 3 orders of magnitude higher than the affinity of CD4 for its putative endogenous ligand, the MHC class II antigens. Indeed, to date, a specific physical association between monomeric CD4 and class II antigens has not been demonstrated.

In response to bacterial or other particle infection, the host organism usually produces serum antibodies that bind to specific proteins or carbohydrates on the bacterial or particle surface, coating the bacteria. This antibody coat on the bacterium or other particle stimulates cytotoxicity by Fc-receptor-bearing lymphoid cells by antibody-dependent cellular toxicity (ADCC). Other serum proteins, collectively called complement (C), bind to antibody-coated targets, and also can coat foreign particles nonspecifically. They cause cell death by lysis, or stimulate ingestion by binding to specific receptors on the macrophage called complement receptors. See Darnell J. *et al.*, in Molecular Cell Biology, Scientific American Books, pp. 641 and 1087 (1986).

The most effective complement activating classes of human Ig are IgM and IgG1. The complement system consists of 14 proteins that, acting in order, cause lysis of cells. Nearly all of the C proteins exist in normal serum as inactive precursors. When activated, some

become highly specific proteolytic enzymes whose substrate is the next protein in a sequential chain reaction.

The entire C sequence can be triggered by either of two initiation pathways. In one (the classic pathway), Ab-Ag complexes bind and activate C1, C4 and C2 to form a C3-splitting enzyme. In the second pathway, polysaccharides commonly on the surface of many bacteria and fungi bind with trace amounts of a C3 fragment and then with two other proteins (factor B and properdin) to form another C3-splitting enzyme. Once C3 is split by either pathway, the way is open for the remaining sequence of steps which lead to cell lysis. See Davis, B.D., *et al.*, In Microbiology, 3rd ed., Harper and Row, Philadelphia, PA, pp. 452-466 (1980).

A number of workers have disclosed methods for preparing hybrid proteins. For example, Murphy, United States Patent 4,675,382 (1987), discloses the use of recombinant DNA techniques to make hybrid protein molecules by forming the desired fused gene coding for a hybrid protein of diphtheria toxin and a polypeptide ligand such as a hormone, followed by expression of the fused gene.

Many workers have prepared monoclonal antibodies (Mabs) by recombinant DNA techniques. Monoclonal antibodies are highly specific well-characterized molecules in both primary and tertiary structure. They have been widely used for in vitro immunochemical characterization and quantitation of antigens. Genes for heavy and light chains have been introduced into appropriate hosts and expressed, followed by reaggregation of the individual chains into functional antibody molecules (see, for example, Munro, Nature 312:597 (1984); Morrison, S.L., Science 229:1202 (1985); Oi *et al.*, Biotechniques 4:214 (1986); Wood *et al.*, Nature 314:446-449 (1985)). Light- and heavy-chain variable regions have been cloned and expressed in foreign hosts wherein they maintained their binding ability (Moore *et al.*, European Patent Application 0088994 (published September 21, 1983)).

Chimeric or hybrid antibodies have also been prepared by recombinant DNA techniques. Oi and Morrison, Biotechniques 4:214 (1986)

describe a strategy for producing such chimeric antibodies which include a chimeric human IgG anti-leu3 antibody.

Gascoigne, N.R.J., et al., Proc. Natl. Acad. Sci. (USA) 84:2936-2940 (1987) disclose the preparation of a chimeric gene construct containing a T-cell receptor α -chain variable (V) domain and the constant (C) region coding sequence of an immunoglobulin γ 2a molecule. Cells transfected with the chimeric gene synthesize a protein product that expresses immunoglobulin and T-cell receptor antigenic determinants as well as protein A binding sites. This protein associates with a normal λ chain to form an apparently normal tetrameric (H_2L_2 , where H=heavy and L=light) immunoglobulin molecule that is secreted.

Sharon, J., et al., Nature 309:54 (1984), disclose construction of a chimeric gene encoding the variable (V) region of a mouse heavy chain specific for the hapten azophenylarsonate and the constant (C) region of a mouse kappa light chain ($V_H C_K$). This gene was introduced into a mouse myeloma cell line. The chimeric gene was expressed to give a protein which associated with light chains secreted from the myeloma cell line to give an antibody molecule specific for azophenylarsonate.

Morrison, Science 229:1202 (1985), discloses that variable light- or variable heavy-chain regions can be attached to a non-Ig sequence to create fusion proteins. This article states that the potential uses for the fusion proteins are three: (1) to attach antibody specifically to enzymes for use in assays; (2) to isolate non-Ig proteins by antigen columns; and (3) to specifically deliver toxic agents.

Recent techniques for the stable introduction of immunoglobulin genes into myeloma cells (Banerji, J., et al., Cell 33:729-740 (1983); Potter, H., et al., Proc. Natl. Acad. Sci. (USA) 81:7161-7165 (1984)), coupled with detailed structural information, have permitted the use of in vitro DNA methods such as mutagenesis, to generate recombinant antibodies possessing novel properties.

PCT Application WO87/02671 discloses methods for producing genetically engineered antibodies of desired variable region specificity and constant region properties through gene cloning and expression of light and heavy chains. The mRNA from cloned hybridoma B cell lines which produce monoclonal antibodies of desired specificity is isolated for cDNA cloning. The generation of light and heavy chain coding sequences is accomplished by excising the cloned variable regions and ligating them to light or heavy chain module vectors. This gives cDNA sequences which code for immunoglobulin chains. The lack of introns allows these cDNA sequences to be expressed in prokaryotic hosts, such as bacteria, or in lower eukaryotic hosts, such as yeast.

The generation of chimeric antibodies in which the antigen-binding portion of the immunoglobulin is fused to other moieties has been demonstrated. Examples of non-immunoglobulin genes fused to antibodies include Staphylococcus aureus nuclease, the mouse oncogene c-myc, and the Klenow fragment of E. coli DNA polymerase I (Neuberger, M.S., et al., Nature 312:604-612 (1984); Neuberger, M.S., Trends in Biochemical Science, 347-349 (1985)). European Patent Application 120,694 discloses the genetic engineering of the variable and constant regions of an immunoglobulin molecule that is expressed in E. coli host cells. It is further disclosed that the immunoglobulin molecule may be synthesized by a host cell with another peptide moiety attached to one of the constant domains. Such peptide moieties are described as either cytotoxic or enzymatic. The application and the examples describe the use of a lambda-like chain derived from a monoclonal antibody which binds to 4-hydroxy-3-nitrophenyl (NP) haptens.

European Patent Application 125,023 relates to the use of recombinant DNA techniques to produce immunoglobulin molecules that are chimeric or otherwise modified. One of the uses described for these immunoglobulin molecules is for whole-body diagnosis and treatment by injection of the antibodies directed to specific target tissues. The presence of the disease can be determined by attaching a

suitable label to the antibodies, or the diseased tissue can be attacked by carrying a suitable drug with the antibodies. The application describes antibodies engineered to aid the specific delivery of an agent as "altered antibodies."

PCT Application W083/171533 describes chimeric antibodies wherein the variable region of an immunoglobulin molecule is linked to a portion of a second protein which may comprise the active portion of an enzyme.

Boulianne et al., Nature 312:643 (1984) constructed an immunoglobulin gene in which the DNA segments that encode mouse variable regions specific for the hapten trinitrophenol (TNP) are joined to segments that encode human mu and kappa regions. These chimeric genes were expressed to give functional TNP-binding chimeric IgM.

Morrison et al., P.N.A.S. (USA) 81:6851 (1984), disclose a chimeric molecule utilizing the heavy-chain variable region exons of an anti-phosphoryl choline myeloma protein G, which were joined to the exons of either human kappa light-chain gene. The genes were transfected into mouse myeloma cell lines, generating transformed cells that produced chimeric mouse-human IgG with antigen-binding function.

Despite the progress that has been achieved on determining the mechanism of HIV infection, a need continues to exist for methods of treating HIV viral infections.

SUMMARY OF THE INVENTION

The invention relates to a gene comprising a DNA sequence which encodes a fusion protein comprising 1) CD4, or a fragment thereof which binds to HIV gp120, and 2) an immunoglobulin light or heavy chain; wherein said CD4 or HIV gp120-binding fragment thereof replaces the variable region of the light or heavy immunoglobulin chain.

The invention also relates to vectors containing the gene of the invention and hosts transformed with the vectors.

The invention also relates to a method of producing a fusion protein comprising CD4, or fragment thereof which binds to HIV gp120, and an immunoglobulin light or heavy chain, wherein the variable region of the immunoglobulin light or heavy chain has been substituted with CD4, or HIV gp120-binding fragment thereof, which comprises:

cultivating in a nutrient medium under protein producing conditions, a host strain transformed with the vector containing the gene of the invention, said vector further comprising expression signals which are recognized by said host strain and direct expression of said fusion protein, and recovering the fusion protein so produced.

The invention also relates to a fusion protein comprising CD4, or fragment thereof which is capable of binding to HIV gp120, fused at the C-terminus to a second protein which comprises an immunoglobulin light or heavy chain, wherein the variable region of said light or heavy chain is substituted with CD4 or a HIV gp120 binding fragment thereof.

The invention also relates to an immunoglobulin-like molecule comprising the fusion protein of the invention together with an immunoglobulin light or heavy-chain, wherein said immunoglobulin like molecule binds HIV gp120.

The IgG1 fusion proteins and immunoglobulin-like molecules may be useful for both complement-mediated and cell-mediated (ADCC) immunity, while the IgM fusion proteins are useful principally through complement-mediated immunity.

The invention also relates to a complex between the fusion proteins and immunoglobulin-like molecule of the invention and HIV gp120.

The invention also relates to a method for treating HIV or SIV infections comprising administering the fusion protein or immunoglobulin-like molecule of the invention to an animal.

The invention further relates to a method for detecting HIV gp120 in a sample comprising contacting a sample suspected of containing HIV

or gp120 with the fusion protein or immunoglobulin-like molecule of the invention, and detecting whether a complex has formed.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention is directed to a protein gene which comprises

- 1) a DNA sequence which codes for CD4, or fragment thereof which binds to HIV gp120, fused to
- 2) a DNA sequence which encodes an immunoglobulin heavy chain.

Preferably, the antibody has effector function.

The invention is also directed to a protein gene which comprises

- 1) a DNA sequence which codes for CD4, or fragment thereof which binds to HIV gp120, fused to
- 2) a DNA sequence which encodes an immunoglobulin light chain; wherein said sequence which codes for CD4, or HIV gp120-binding fragment thereof, replaces the variable region of the light immunoglobulin chain.

The invention is also directed to the expression of these novel fusion proteins in transformed hosts and the use thereof to treat and diagnose HIV infections. In particular, the invention relates to expressing said genes in mammalian hosts which express complementary light or heavy chain immunoglobulins to give immunoglobulin-like molecules which have antibody effector function and also bind to HIV or SIV gp120.

The term "antibody effector function" as used herein denotes the ability to fix complement or to activate ADCC.

The fusion proteins and immunoglobulin-like molecules may be administered to an animal for the purpose of treating HIV or SIV infections. By the terms "HIV infections" is intended the condition of having AIDS, AIDS related complex (ARC) or where an animal harbors the AIDS virus, but does not exhibit the clinical symptoms of AIDS or

ARC. By the terms "SIV infections" is intended the condition of being infected with simian immunodeficiency virus.

By the term "animal" is intended all animals which may derive benefit from the administration of the fusion proteins and immunoglobulin-like molecules of the invention. Foremost among such animals are humans, however, the invention is not intended to be so limited.

By the term "fusion protein" is intended a fused protein comprising CD4, or fragment thereof which is capable of binding to gp120, linked at its C-terminus to an immunoglobulin chain wherein a portion of the N-terminus of the immunoglobulin is replaced with CD4. In general, that portion of immunoglobulin which is deleted is the variable region. The fusion proteins of the invention may also comprise immunoglobulins where more than just the variable region has been deleted and replaced with CD4 or HIV gp120 binding fragment thereof. For example, the V_H and CH1 regions of an immunoglobulin chain may be deleted. Preferably, any amount of the N-terminus of the immunoglobulin heavy chain can be deleted as long as the remaining fragment has antibody effector function. The minimum sequence required for binding complement encompasses domains CH2 and CH3. Joining of Fc portions by the hinge region is advantageous for increasing the efficiency of complement binding.

The CD4 portion of the fusion protein may comprise the complete CD4 sequence, the 370 amino acid extracellular region and the membrane spanning domain, or the extracellular region. The fusion protein may comprise fragments of the extracellular region obtained by cutting the DNA sequence which encodes CD4 at the BspM1 site at position 514 or the PvuII site at position 629 (see Table 1) to give nucleotide sequences which encode CD4 fragments which retain binding to gp120. In general, any fragment of CD4 may be used as long as it retains binding to gp120.

Where the fusion protein comprises an immunoglobulin light chain, it is necessary that no more of the Ig chain be deleted than is necessary to form a stable complex with a heavy chain Ig. In particu-

lar, the cysteine residues necessary for disulfide bond formation must be preserved on both the heavy and light chain moieties.

When expressed in a host, e.g., a mammalian cell, the fusion protein may associate with other light or heavy Ig chains secreted by the cell to give a functioning immunoglobulin-like molecule which is capable of binding to gp120. The gp120 may be in solution, expressed on the surface of infected cells, or may be present on the surface of the HIV virus itself. Alternatively, the fusion protein may be expressed in a mammalian cell which does not secrete other light or heavy Ig chains. When expressed under these conditions, the fusion protein may form a homodimer.

Genomic or cDNA sequences may be used in the practice of the invention. Genomic sequences are expressed efficiently in myeloma cells, since they contain native promoter structures.

The constant regions of the antibody cloned and used in the chimeric immunoglobulin-like molecule may be derived from any mammalian source. The constant regions may be complement binding or ADCC active. However, preliminary work (see Examples) indicates that the fusion proteins of the invention may mediate HIV or SIV infected cell death by an ADCC or complement-independent mechanism. The constant regions may be derived from any appropriate isotype, including IgG1, IgG3, or IgM.

The joining of various DNA fragments, is performed in accordance with conventional techniques, employing blunt-ended or staggered-ended termini for ligation, restriction enzyme digestion to provide appropriate termini, filling in of cohesive ends as appropriate, alkali and phosphatase treatment to avoid undesirable joining, and ligation with appropriate ligases. The genetic construct may optionally encode a leader sequence to allow efficient expression of the fusion protein. For example, the leader sequence utilized by Maddon et al., Cell 42:93-104 (1985) for the expression of CD4 may be used.

For cDNA, the cDNA may be cloned and the resulting clone screened, for example, by use of a complementary probe or by assay for

expressed CD4 using an antibody as disclosed by Dalgleish et al., Nature 312:763-766 (1984); Klatzmann et al., Immunol. Today 7:291-297

(1986); McDougal et al., J. Immunol. 135:3151-3162 (1985); and McDougal, J. et al., J. Immunol. 137:2937-2944 (1986).

To express the fusion hybrid protein, transcriptional and translational signals recognized by an appropriate host element are necessary. Eukaryotic hosts which may be used include mammalian cells capable of culture in vitro, particularly leukocytes, more particularly myeloma cells or other transformed or oncogenic lymphocytes, e.g., EBV-transformed cells. Alternatively, non-mammalian cells may be employed, such as bacteria, fungi, e.g., yeast, filamentous fungi, or the like.

Preferred hosts for fusion protein production are mammalian cells, grown in vitro in tissue culture or in vivo in animals. Mammalian cells provide post translational modification to immunoglobulin protein molecules which provide for correct folding and glycosylation of appropriate sites. Mammalian cells which may be useful as hosts include cells of fibroblast origins such as VERO or CHO-K1 or cells of lymphoid origin, such as the hybridoma SP2/0-AG14 or the myeloma P3x63Sgh, and their derivatives. For the purpose of preparing an immunoglobulin-like molecule, a plasmid containing a gene which encodes a heavy chain immunoglobulin, wherein the variable region has been replaced with CD4 or fragment thereof which binds to gp120, may be introduced, for example, into J558L myeloma cells, a mouse plasmacytoma expressing the lambda-1 light chain but which does not express a heavy chain (see Oi et al., P.N.A.S. (USA) 80:825-829 (1983)). Other preferred hosts include COS cells, BHK cells and hepatoma cells.

The constructs may be joined together to form a single DNA segment or may be maintained as separate segments, by themselves or in conjunction with vectors.

Where the fusion protein is not glycosylated, any host may be used to express the protein which is compatible with replicon and control sequences in the expression plasmid. In general, vectors containing replicon and control sequences are derived from species compatible with a host cell are used in connection with the host. The vector ordinarily carries a replicon site, as well as specific genes which are capable of providing phenotypic selection in transformed cells. The expression of the fusion protein can also be placed under control with other regulatory sequences which may be homologous to the organism in its untransformed state. For example, lactose-dependent E. coli chromosomal DNA comprises a lactose or lac operon which mediates lactose utilization by elaborating the enzyme beta-galactosidase. The lac control elements may be obtained from bacterial phage lambda plac5, which is infective for E. coli. The lac promoter-operator system can be induced by IPTG.

Other promoters/operator systems or portions thereof can be employed as well. For example, colicin E1, galactose, alkaline phosphatase, tryptophan, xylose, tax, and the like can be used.

For mammalian hosts, several possible vector systems are available for expression. One class of vectors utilize DNA elements which are derived from animal viruses such as bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (RSV, MMTV or MOMLV), or SV40 virus. Cells which have stably integrated the DNA into their chromosomes may be selected by introducing one or more markers which allow selection of transfected host cells. The marker may provide for prototrophy to an auxotrophic host, biocide resistance, e.g., antibiotics, or heavy metals such as copper or the like. The selectable marker gene can be either directly linked to the DNA sequences to be expressed, or introduced into the same cell by cotransformation. Additional elements may also be needed for optimal synthesis of mRNA. These elements may include splice signals, as well as transcriptional promoters, enhancers, and termination signals. The

cDNA expression vectors incorporating such elements includes those described by Okayama, H., Mol. Cell. Biol., 3:280 (1983) and others.

Once the vector or DNA sequence containing the constructs has been prepared for expression, the DNA constructs may be introduced to an appropriate host. Various techniques may be employed, such as protoplast fusion, calcium phosphate precipitation, electroporation or other conventional techniques. After the fusion, the cells are grown in media and screened for the appropriate activity. Expression of the gene(s) results in production of the fusion protein. This expressed fusion protein may then be subject to further assembly to form the immunoglobulin-like molecule.

The host cells for immunoglobulin production may be immortalized cells, primarily myeloma or lymphoma cells. These cells may be grown in appropriate nutrient medium in culture flasks or injected into a synergistic host, e.g., mouse or a rat, or immunodeficient host or host site, e.g., nude mouse or hamster pouch. In particular, the cells may be introduced into the abdominal cavity of an animal to allow production of ascites fluid which contains the immunoglobulin-like molecule. Alternatively, the cells may be injected subcutaneously and the chimeric antibody is harvested from the blood of the host. The cells may be used in the same manner as hybridoma cells. See Diamond et al., N. Eng. J. Med. 304:1344 (1981), and Kennatt, McKearn and Bechtol (Eds.), Monoclonal Antibodies: Hybridomas: -- A New Dimension in Biologic Analysis, Plenum, 1980.

The fusion proteins and immunoglobulin-like molecules of the invention may be isolated and purified in accordance with conventional conditions, such as extraction, precipitation, chromatography, affinity chromatography, electrophoresis or the like. For example, the IgG1 fusion proteins may be purified by passing a solution through a column which contains immobilized protein A or protein G which selectively binds the Fc portion of the fusion protein. See, for example, Reis, K.J., et al., J. Immunol. 132:3098-3102 (1984); PCT Application, Publication No. W087/00329. The chimeric antibody may

the be eluted by treatment with a chaotropic salt or by elution with aqueous acetic acid (1 M).

Alternatively the fusion proteins may be purified on anti-CD4 antibody columns, or on anti-immunoglobulin antibody columns.

In one embodiment of the invention, cDNA sequences which encode CD4, or a fragment thereof which binds gp120, may be ligated into an expression plasmid which codes for an antibody wherein the variable region of the gene has been deleted. Methods for the preparation of genes which encode the heavy or light chain constant regions of immunoglobulins are taught, for example, by Robinson, R. et al., PCT Application, Publication No. WO87-02671.

Preferred immunoglobulin-like molecules which contain CD4, or fragments thereof, contain the constant region of an IgM, IgG1 or IgG3 antibody which binds complement at the Fc region.

The fusion protein and immunoglobulin-like molecules of the invention may be used for the treatment of HIV viral infections. The fusion protein complexes to gp120 which is expressed on infected cells. Although the inventor is not bound by a particular theory, it appears that the Fc portion of the hybrid fusion protein may bind with complement, which mediates destruction of the cell. In this manner, infected cells are destroyed so that additional viral particle production is stopped.

For the purpose of treating HIV infections, the fusion protein or immunoglobulin-like molecule of the invention may additionally contain a radiolabel or therapeutic agent which enhances destruction of the HIV particle or HIV-infected cell.

Examples of radioisotopes which can be bound to the fusion protein or immunoglobulin-like molecule of the invention for use in HIV-therapy are ^{125}I , ^{131}I , ^{90}Y , ^{67}Cu , ^{217}Bi , ^{211}At , ^{212}Pb , ^{47}Sc , and ^{109}Pd . Optionally, a label such as boron can be used which emits α and β particles upon bombardment with neutron radiation.

For in vivo diagnosis radionucleotides may be bound to the fusion protein or immunoglobulin-like molecule of the invention either directly or by using an intermediary functional group. An intermediary group which is often used to bind radioisotopes, which exist as metallic cations, to antibodies is diethylenetriaminepentaacetic

acid (DTPA). Typical examples of metallic cations which are bound in this manner are ^{99m}Tc , ^{123}I , ^{111}In , ^{131}I , ^{97}Ru , ^{67}Cu , ^{67}Ga , and ^{68}Ga .

Moreover, the fusion protein and immunoglobulin-like molecule of the invention may be tagged with an NMR imaging agent which include paramagnetic atoms. The use of an NMR imaging agent allows the in vivo diagnosis of the presence of and the extent of HIV infection within a patient using NMR techniques. Elements which are particularly useful in this manner are ^{157}Gd , ^{55}Mn , ^{162}Dy , ^{52}Cr , and ^{56}Fe .

Therapeutic agents may include, for example, bacterial toxins such as diphtheria toxin, or ricin. Methods for producing fusion proteins comprising fragment A of diphtheria toxin are taught in U.S. Patent 4,675,382 (1987). Diphtheria toxin contains two polypeptide chains. The B chain binds the toxin to a receptor on a cell surface. The A chain actually enters the cytoplasm and inhibits protein synthesis by inactivating elongation factor 2, the factor that translocates ribosomes along mRNA concomitant with hydrolysis of ETP. See Darnell, J., et al., in Molecular Cell Biology, Scientific American Books, Inc., page 662 (1986). Alternatively, a fusion protein comprising ricin, a toxic lectin, may be prepared.

Introduction of the chimeric molecules by gene therapy may also be contemplated, for example, using retroviruses or other means to introduce the genetic material encoding the fusion proteins into suitable target tissues. In this embodiment, the target tissues having the cloned genes of the invention may then produce the fusion protein in vivo.

The dose ranges for the administration of the fusion protein or immunoglobulin-like molecule of the invention are those which are

large enough to produce the desired effect whereby the symptoms of HIV or SIV infection are ameliorated. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of disease in the patient, counterindications, if any, immune tolerance and other such variables, to be adjusted by the individual physician. Dosage can vary from .01 mg/kg to 50 mg/kg, preferably 0.1 mg/kg to 1.0 mg/kg, of the immunoglobulin-like molecule in one or more administrations daily, for one or several days. The immunoglobulin-like molecule can be administered parenterally by injection or by gradual perfusion over time. They can be administered intravenously, intraperitoneally, intramuscularly, or subcutaneously.

Preparations for parenteral administration include sterile or aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases and the like. See, generally, Remington's Pharmaceutical Science, 16th Ed., Mack Eds., 1980.

The invention also relates to a method for preparing a medicament or pharmaceutical composition comprising the components of the invention, the medicament being used for therapy of HIV or SIV infection in animals.

The detection and quantitation of antigenic substances and biological samples frequently utilized immunoassay techniques. These

techniques are based upon the formation of the complex between the antigenic substance, e.g., gp120, being assayed and an antibody or antibodies in which one or the other member of the complex may be detectably labeled. In the present invention, the immunoglobulin-like molecule or fusion protein may be labeled with any conventional label.

Thus, the hybrid fusion protein or immunoglobulin-like molecule of the invention can also be used in assay for HIV or SIV viral infection in a biological sample by contacting a sample, derived from an animal suspected of having an HIV or SIV infection, with the fusion protein or immunoglobulin-like molecule of the invention, and detecting whether a complex with gp120, either alone or on the surface of an HIV-infected cell, has formed.

For example, a biological sample may be treated with nitro-cellulose, or other solid support which is capable of immobilizing cells, cell particles or soluble protein. The support may then be washed with suitable buffers followed by treatment with the fusion protein which may be detectably labeled. The solid phase support may then be washed with the buffer a second time to remove unbound fusion protein and the label on the fusion protein detected.

In carrying out the assay of the present invention on a sample containing gp120, the process comprises:

a) contacting a sample suspected containing gp120 with a solid support to effect immobilization of gp120, or cell which expresses gp120 on its surface;

b) contacting said solid support with the detectably labeled immunoglobulin-like molecule or fusion protein of the invention;

c) incubating said detectably labeled immunoglobulin-like molecule with said support for a sufficient amount of time to allow the immunoglobulin-like molecule or fusion protein to bind to the immobilized gp120 or cell which expresses gp120 on its surface;

d) separating the solid phase support from the incubation mixture obtained in step c); and

e) detecting the bound immunoglobulin-like molecule or fusion protein and thereby detecting and quantifying gp120.

Alternatively, labeled immunoglobulin-like molecule (or fusion protein) -gp120 complex in a sample may be separated from a reaction mixture by contacting the complex with an immobilized antibody or protein which is specific for an immunoglobulin or, e.g., protein A, protein G, anti-IgM or anti-IgG antibodies. Such anti-immunoglobulin antibodies may be monoclonal or polyclonal. The solid support may then be washed with suitable buffers to give an immobilized gp120-labeled immunoglobulin-like molecule antibody complex. The label on the fusion protein may then be detected to give a measure of endogenous gp120 and, thereby, the presence of HIV.

This aspect of the invention relates to a method for detecting HIV or SIV viral infection in a sample comprising

- (a) contacting a sample suspected of containing gp120 with a fusion protein or immunoglobulin-like molecule comprising CD4, or fragment thereof which binds to gp120, and the Fc portion of an immunoglobulin chain,
- (b) detecting whether a complex is formed.

The invention also relates to a method of detecting gp120 in a sample, further comprising

- (c) contacting the mixture obtained in step (a) with an Fc binding molecule, such as an antibody, protein A, or protein G, which is immobilized on a solid phase support and is specific for the hybrid fusion protein, to give a gp120 fusion protein-immobilized antibody complex
- (d) washing the solid phase support obtained in step (c) to remove unbound fusion protein,
- (e) and detecting the label on the hybrid fusion protein.

Of course, the specific concentrations of detectably labeled immunoglobulin-like molecule (or fusion protein) and gp120, the temperature and time of incubation, as well as other assay conditions

may be varied, depending on various factors including the concentration of gp120 in the sample, the nature of the sample, and the like. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

Other such steps as washing, stirring, shaking, filtering and the like may be added to the assays as is customary or necessary for the particular situation.

One of the ways in which the immunoglobulin-like molecule or fusion protein of the present invention can be detectably labeled is by linking the same to an enzyme. This enzyme, in turn, when later exposed to its substrate, will react with the substrate in such a manner as to produce a chemical moiety which can be detected as, for example, by spectrophotometric, fluorometric or by visual means. Enzymes which can be used to detectably label the immunoglobulin-like molecule or fusion protein of the present invention include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-V-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-VI-phosphate dehydrogenase, glucoamylase and acetylcholine esterase.

The immunoglobulin like molecule or fusion protein of the present invention may also be labeled with a radioactive isotope which can be determined by such means as the use of a gamma counter or a scintillation counter or by autoradiography. Isotopes which are particularly useful for the purpose of the present invention are: ^3H , ^{125}I , ^{131}I , ^{32}P , ^{35}S , ^{14}C , ^{51}Cr , ^{36}Cl , ^{57}Co , ^{58}Co , ^{59}Fe and ^{75}Se .

It is also possible to label the immunoglobulin-like molecule or fusion protein with a fluorescent compound. When the fluorescently labeled immunoglobulin-like molecule is exposed to light of the proper wave length, its presence can then be detected due to the fluorescence

of the dye. Among the most commonly used fluorescent labelling compounds are fluorescein isothiocyanate, rhodamine, phycoerytherin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

The immunoglobulin-like molecule or fusion protein of the invention can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu , or others of the lanthanide series. These metals can be attached to the immunoglobulin-like molecule or fusion protein using such metal chelating groups as diethylenetriaminepenta-acetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

The immunoglobulin-like molecule or fusion protein of the present invention also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged immunoglobulin-like molecule or fusion protein is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

Likewise, a bioluminescent compound may be used to label the immunoglobulin-like molecule or fusion protein of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

Detection of the immunoglobulin-like molecule or fusion protein may be accomplished by a scintillation counter, for example, if the detectable label is a radioactive gamma emitter, or by a fluorometer, for example, if the label is a fluorescent material. In the case of an enzyme label, the detection can be accomplished by colorimetric methods which employ a substrate for the enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic

reaction of a substrate in comparison with similarly prepared standards.

The assay of the present invention is ideally suited for the preparation of a kit. Such a kit may comprise a carrier means being compartmentalized to receive in close confinement therewith one or more container means such as vials, tubes and the like, each of said container means comprising the separate elements of the immunoassay. For example, there may be a container means containing a solid phase support, and further container means containing the detectably labeled immunoglobulin-like molecule or fusion protein in solution. Further container means may contain standard solutions comprising serial dilutions of analytes such as gp120 or fragments thereof to be detected. The standard solutions of these analytes may be used to prepare a standard curve with the concentration of gp120 plotted on the abscissa and the detection signal on the ordinate. The results obtained from a sample containing gp120 may be interpolated from such a plot to give the concentration of gp120.

The immunoglobulin-like molecule or fusion protein of the present invention can also be used as a stain for tissue sections. For example, a labeled immunoglobulin-like molecule comprising CD4 or fragment thereof which binds to gp120 may be contacted with a tissue section, e.g., a brain biopsy specimen. This section may then be washed and the label detected.

The following examples are illustrative, but not limiting the method and composition of the present invention. Other suitable modifications and adaptations which are obvious to this skill in the art are within the spirit and scope of this invention.

EXAMPLES

Example 1: Preparation of CD4-Ig cDNA Constructs

The extracellular portion of the CD4 molecule (See Madden, P.J., et al., Cell 42:93-104 (1985)) was fused at three locations in a human IgG1 heavy chain constant region gene by means of a synthetic splice donor linker molecule. To exploit the splice donor linker, a BamHI linker having the sequence CGCGGATCCGCG was first inserted at amino acid residue 395 of the CD4 precursor sequence (nucleotide residue 1295). A synthetic splice donor sequence

GATCCCGAGGGTGAGTACTA

GGCTCCCACTCATGATTCGA

bounded by BamHI and HindIII complementary ends was created and fused to the HindIII site in the intron preceding the CH1 domain, to the EspI site in the intron preceding the hinge domain, and to the BstI site preceding the CH2 domain of the IgG1 genomic sequence. Assembly of the chimeric genes by ligation at the BamHI site afforded molecules in which either the variable (V) region, the V+CH1 regions, or the V, CH1 and hinge regions were replaced by CD4. In the last case, the chimeric molecule is expected to form a monomer structure, while in the former, a dimeric molecule is expected.

On such genetic construct which contains the DNA sequence which encodes CD4 linked to human IgG1 at the Hind3 site upstream of the CH1 region (fusion protein CD4Hyl) is depicted in Table 1. The plasmid containing this genetic construct (pCD4Hyl) has been deposited in E. coli (MC1061/P3) at the American Type Culture Collection (ATCC) under the terms of the Budapest Treaty and given accession number 67611.

A second genetic construct which contains the DNA sequence which encodes CD4 linked to human IgG1 at the Esp site upstream of the hinge region (fusion protein CD4Eyl) is depicted in Table 2. The plasmid containing this genetic construct (pCD4Eyl) has been deposited in E. coli (MC1061/P3) at the ATCC under the terms of the Budapest Treaty and given accession number 67610.

A third genetic construct which contains the DNA sequence which encodes CD4 linked to human IgM at the Hst2 site upstream of the CH1 region (fusion protein CD4Myl) is depicted in Table 3. The plasmid

-24-

containing this genetic construct (pCD4M_μ) has been deposited in E. coli (MC1061/F3) at the ATCC under the terms of the Budapest Treaty and given accession number 67609.

A fourth genetic construct which contains the DNA sequence which encodes CD4 linked to human IgM at the Pst site upstream of the CH2 region (fusion protein CD4P_μ) is depicted in Table 4. The plasmid containing this genetic construct (pCD4P_μ) has been deposited in E. coli (MC1061/P3) at the ATCC under the terms of the Budapest Treaty and given accession number 67608.

A fifth genetic construct which contains the DNA sequence which encodes CD4 linked to human IgG1 at the BanI site downstream from the hinge region (fusion protein CD4B_γ1) is depicted in Table 5.

Two similar constructs were prepared from the human IgM heavy chain constant region by fusion with the introns upstream of the μ CH1 and CH2 domains at an MstII site and a PstI site respectively. The fusions were made by joining the PstI site of the CD4/IgG1 construct fused at the Esp site in IgG1 gene to the MstII and Pst sites in the IgM gene. In the first instance, this was performed by treatment of the Pst end with T4 DNA Polymerase and the MstII end with E. coli DNA Polymerase, followed by ligation; and in the second instance, by ligation alone.

Immunoprecipitation of the fusion proteins with a panel of monoclonal antibodies directed against CD4 epitopes showed that all of the epitopes were preserved. A specific high affinity association is demonstrated between the chimeric molecules and HIV envelope proteins expressed on the surface of cells transfected with an attenuated (reverse transcriptase deleted) proviral construct.

-27-

B
S
P
M
1

S
T
Y
1

481 GATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTGACCCTGACCTTGG

CTAACTGACGGTTGAGACTGTGGGTGGACGAAGTCCCCGTCTCGGACTGGGACTGGAACC 540

L T A N S D T H L L Q G Q S L T L T L E -

B BS
BS SC
AP TR
N1 NF
22 11
/ /

D
D
E
1

M
N
L
1

H
I
N
F
1

S
T
Y
1

541 AGAGCCCCCTGGTAGTAGCCCCCTCAGTGCAATGTAGGAGTCCAAGGGGTAAAAACATAC

TCTCGGGGGGACCATCATCGGGAGTCACGTTACATCCTCAGGTTCCCCATTTTGTATG 600

S P P G S S P S V Q C R S P R G K N I Q -

N
BBH S B BS
A BSSGSC S B N SC
L APTIAR T A L TR
U N1NACF X N A NF
2 11 122 1 221111 1 1 4 11
// /

601 AGGGGGGGAAGACCCCTCTCCGTGTCTCAGCTGGAGCTCCAGGATAGTGGCACCTGGACAT

TCCCCCCTTCTGGGAGAGGCACAGAGTCGACCTCGAGGTCCTATCACCGTGGACCTGTA 660

G G K T L S V S Q L E L R D S G T W T C -

N
NS
LP
AH
31
/

M
B
O
2

NM A
HA L
EE U
11 1

661 GCACTGTCTTGACAACCAGAAGAGGTGGAGTTCAAAATAGACATCGTGGTCTAGCTT

CGTGACAGAAGCTCTTGGTCTTCTCCACCTCAAGTTTTATCTGTAGCACCACGATCGAA 720

T V L Q N Q K K V E F K I D I V V L A F -

MS	M	M
AT	N	N
EU	L	L
31	1	1

721 TCCAGAAGCCCTCCAGCATAGTCTATAAGAAAGAGGGGAACAGGTGGAGTTCTCCTTCC
 AGGTCTTCCGGAGGTCTGATCAGATATTCTTTCTCCCCCTTGTCCACCTCAAGAGCAAGG 780

Q K A S S I V Y K K E G E Q V E F S F P -

	A	A	M
	L	L	N
	U	U	L
	1	1	1

781 CACTCGCCTTTACAGTTGAAAAGCTGACGGGCACTGGCGAGCTGTGGTGGCAGGCGGAGA
 GTGAGCGGAAATGTCAACTTTTCGACTGCCCGTCACCGCTCGACACCACCGTCCGCTCT 840

L A F T V E K L T G S G E L W W Q A E R -

	P	S	
H	M	F	A
P	N	L	U
H	L	M	3
1	1	11	A
			2

841 GGGCTTCTCTCCAAGTCTTGGATCACCTTTGACCTGAAGAACAAGGAAGTGTETGTAA
 CCCGAAGGAGGAGGTTGAGAACCTAGTGGAACTGGACTTCTTGTTCCTTCACAGACATT 900

A S S S K S W I T F D L K N K E V S V K -

B	BS	PS	
SM	SCADNPAD	A	AH
TA	TRVRLUJD	L	LP
EE	NFAAAM9E	U	UH
23	11224161	1	11

901 AACGGGTTACCCAGGACCCCTAAGCTCCAGATGGGCAAGAAGCTCCCGCTCCACCTCACCC
 TTGCCCAATGGGTCTCGGATTGAGGTCTACCCGTTCTTCGAGGGCGAGGTGGAGTGGG 960

R V T Q D P K L Q M G K K L P L H L T L -

-29-

BS
M SC HS
N TR AT
L NF EU
1 11 31

D
D
E
1

M H
N P
L H
1 1

BSS
SCAHN
TRUAN
NF9EL
11631

961 TGGCCCAGGCCTTGCCCTCAGTATGCTGGCTCTGGAAACCTCACCTGCCCCCTTGAAGCGA 1020
ACGGGGTCCGGAACGGAGTCATACGACCGAGACCTTTGGAGTGGGACCGGGAACCTTCGCT

P Q A L P Q Y A G S G N L T L A L E A K -

S
F
A
N
1

BS
SC
TR
NF
11

H D A
P D L
H E U
1 1 1

1021 AAACAGGAAAGTTGCATCAGGAAGTGAACCTGGTGGTGATGAGAGCCACTCAGCTCCAGA 1080
TTTGTCTTTCAACGTAGTCCTTCACTTGGACCACCACTACTCTCGGTGAGTCGAGGTCT

T G K L H Q E V N L V V M R A T Q L Q K -

M
N
L
1

PS
ADNPA
VRLUU
AAAAM9
224416

S
DF AM
DA LN
EN UL
11 11

DE A
DS L
EP U
11 1

1081 AAAATTTGACCTGTGAGGTGTGGGACCCACCTCCCCTAAGCTGATGCTGAGCTTGAAC 1140
TTTTAACTGGACACTCCACACCCCTGGGTGGAGGGGATTGCACTACGACTCGAAGCTTG

N L T C E V W G P T S P K L M L S L K L -

M
N
L
1

T
A
Q
1

H
P
A
2

M
N
L
1

DM
DS
ET
12

1141 TGGAGAACAAGGAGGCAAAGGTCTCGAAGCGGAGAGCCGGTGTGGGTGCTGAACCTG 1200
ACCTCTTGTTCTCCTCGTTTCCAGAGCTTCGCCCTCTTCGGCCACACCCACGACTTGGGAC

E N X E A K V S K R E K P V W V L N P E -

-30-

				H		PS		H
F	D	M	I	A		ADPA		I
O	D	A	N	V		VRUJ		N
K	E	E	F	A		AAM9		F
1	1	3	1	1		2216		1

1201 AGCGGGGATGTGGCAGTGTCTGCTGAGTGACTCGGGACAGGTCTGCTGGAATCCAACA
 TCCGCCCCCTACACCGTCACAGACGACTCACTGAGCCCTGTCCAGGACGACCTTAGGTTGT 1260

A G M W Q C L L S D S G Q V L L E S N I -

	S	SA	BHF BS		H
ANA	HNCP	SGNMAANDXA		RSD I A	
VLU	PCRA	PIUMULHV		SCD N L	
AA9	AIFL	1ADLH3ADA		AAE D U	
236	2111	21211A421		111 3 1	

1261 TCAAGGTTCTGCCACATGGTCCACCCCGGTGCACGGCATCCCGAGGGTGAGTACTAAG
 AGTTCCAAGACGGGTGTACCAGGTGGGGCCACGTGCGCCTAGGGCTCCCACTCATGATTC 1320

K V L P T W S T P V H A D P E

		BS				B
H	H SC HS	S	M		M D	S
P	A TR AT	T	N		N D	P
H	E NF EU	Y	L		L E	M
1	3 11 31	1	1		1 1	1

1321 CTTTCTGGGGCAGGCCAGGCTGACCTTGCTTTGGGGCAGGGAGGGGGCTAAGGTCAGG
 GAAAGACCCCGTCCGGTCCGGACTGGAACCGAAACCCCGTCCCTCCCCGATTCCACTCC 1380

	B	A	BH		B	P	
BASHBHNN	P	SG		N	BS	F	H
AMPHBAPAL	A	PI		L	AP	L	G
NAMAEHRA	L	1A		A	N1	M	A
121112114	1	21		3	22	1	1

1381 CAGGTGGCGCCAGCAGGTGCACACCCAATGCCCATGAGCCAGACACTGGACGCTGAACC
 GTCCACCOCGCTGTCACGTGTGGGTTACGGGTACTCGGGTCTGTGACCTGCGACTTGG 1440

	F		BS	S	B SS	B S		FN
N	M		SC DNHA		H SMAAHNABSAC			NS
U	N		TR RLAI		H TNUJALPAPLR			UP
D	L		NF AAE9		A NL99EAAN1UF			DB
2	1		11 2436		1 11663412211			22

1441 TCGCGGACAGTTAAGAACCAGGGGCTCTGCGCCTGGGGCCAGCTCTGTCCACACCGC
 AGCGCTGTCAATTCTTGCGTCCCCGGAGACCGGACCCGGTCTGAGACAGGCTGTGGCG 1500
 A49.4.WP 122088

-31-

MS	BNN	F	BSS	BS
AA	ALL	NM	S BMDHNABSAA	SCB
EC	NAA	UN	T BERNALPAPUJ	TRA
32	134	4L	Y VDALEAAN199	NFN
		H1	1 12213412266	111

/ / / / /
 GGTACATGGCACCACCTCTCTTGAGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGG
 1501 ----- 1560
 CCAGTGATCCGTGGTGGAGAGAACGTGGAGGTGGTTCCCGGTAGCCAGAAGGGGGACC
 A S T K G P S V F P L A -

N	BH	B NFS	BS	F	BS
L	M MSG	MSB SNAH	SC	N	SC
A	N NPI	NPB PUJA	TR	U	TR
4	L L1A	L1V B49E	NF	4	NF
	1 121	121 2H63	11	H	11

/ / / / /
 CACCTCTCTCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACT
 1561 ----- 1620
 GTGGGAGGAGGTTCTCGTGGAGACCCCGTGTCCCGGGACCCGACGGACCACTTCTGA
 P S S K S T S G G T A A L G C L V K D Y -

H M	T	H	D	BANHBH	NF	A	BH
P A	T	P	D	AAHABAL	SN	P	SG
A E	H	H	E	NARAEAA	PU	A	PI
2 3	1	1	1	1211124	B4	L	1A
					2H	1	21

/ / / / /
 ACTTCCCCGAACCGGTGACGGTGTCTGGAACCTCAGGCGCCCTGACCAGCGGCGTGACA
 1621 ----- 1680
 TGAAGGGGCTTGGCCACTGCCACAGCACCTTGAGTCCCGGGGACTGGTCCCGCACGTGT
 F P E P V T V S W N S G A L T S G V H T -

S	H	F	B
HNC	DM	N	M SM
PCR	DS	N	N TA
AIF	ET	F	L EE
211	12	1	1

/ / / / /
 CCTTCCCGGCTGTCTACAGTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGC
 1681 ----- 1740
 GGAAGGGCCGACAGGATGTGAGGAGTCTGAGATGAGGGGACTCGTCGACCACTGGCAGC
 F P A V L Q S S G L Y S L S S V V T V P -

B	F B	B	H
SH	N ASM	B MSB	M I
PP	U LTN	A LPB	A N
1H	4 UXL	N A1V	E F
21	H 111	1 421	2 1

/ / / / /
 CCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACA
 1741 ----- 1800
 GCAGGTCTGCGAACCCGTGGGTCTGGATGTAGACGTTGCACTTAGTGTTCGGGTCTGTGT
 S S S L G T Q T Y I C N V N H K P S N T -

-32-

S	M	HM	HM
T	N	AN	PN
Y	L	EL	HL
1	1	31	11

1801 CCAAGGTGCACAAGAAAGTTGGTGAGAGCCACACAGGGAGGGAGGGTGTCTGCTGCAA 1860

GTTCCACCTGTTCTTTCAACCACTCTCGGTCTGTCCCTCCCTCCACAGACGACCT

K V D K K V

	E	BS	SS	F	BS	F
DE	CHH	F	SC	HMCF	N	BSC
DS	DHA	O	TR	PGCRA	U	BTR
EP	4AE	K	NF	AAIFN	4	VNF
11	712	1	11	21111	H	111

1861 GCAGGGCTCAGCGCTCCTGCTGGACGCATCCCGGCTATGCAGCCCCAGTCCAGGGCAGCA 1920

CGTCCGAGTCCGAGGACGGACCTGCGTAGGGCCGATACGTGGGGTCAAGTCCCGTCTGT

S	S	M	MNDM
DBHMHNA	HMNCN	N	NLDB
RBABPLU	PNCRL	L	LAEO
AVEOHA9	ALIFA	1	1312
2132146	21114		

1921 AGGCAGGCCCCGTCTGCTCTTCAACCCGAGCCTCTGCCCGCCCCACTCATGCTCAGGGA 1980

TCCGTCCGGGGCAGACGGAGAAGTGGGCTCGGAGACGGCGGGGTGAGTACGAGTCCCT

BS	P	B	BS
SC	F	M	SC
TR	L	A	TR
NF	M	E	NF
11	1	1	11

1981 GAGGGTCTTCTGGCTTTTTCCAGGCTCTGGGCAGGCACAGGCTAGGTGCCCCAACCCTA 2040

CTCCAGAGACCGAAAAAGGGTCCGAGACCCGTCCGTGTCGATCCACGGGGATTGGGT

S	B	B	B	S
DHA	S	DBS	S	MNC
RAU	P	DAP	P	PCR
AE9	M	EN1	M	AIF
236	1	122	1	211

2041 GGGCTGCACACAAAGGGGCAGGTGCTGGGCTCAGACCTGCCAAGAGCCATATCCGGGAG 2100

CCGGGACGTGTGTTTCCCCGTCCAGGACCCGAGTCTGGACGGTTCTCGGTATAGGCCCTC

-33-

DNPA
RLUJ
AAM9
2416D
D
E
1H
A
E
3D A M
D L N
E U L
1 1 1

2101 GACCTGCCCCCTGACCTAAGCCACCCCAAGGCCAACTCTCCACTCCCTCAGCTCGGA
 CTGGGACGCGGACTGCATTGCGGTGGGTTTCCGGTTTCAGAGGTGAGGGAGTCGAGCCT 2160

 H
I M MM
N N AB
F L EO
1 1 32

 B
P BS
S AP
T N1
1 22

2161 CACCTTCTCTCTCTCCAGATTCCAGTAACTCCCAATCTTCTCTCTGCAGAGCCCAAATCT
 GTGGAAGAGAGGAGGGTCTAAGGTCATTGAGGGTTAGAAGAGAGACGTCTCGGTTTAGA 2220

E P K S -

M
A
E
3
 N
NS
LP
AH
31

 BBS
SSC
PTR
INF
211

 BS
SC HS
TR AT
NF EU
11 31
M
N
L
1

2221 TGTGACAAAACACACATGCCACCGTGCCAGGTAAAGCCAGCCAGGCCTCGCCCTCC
 ACACTGTTTTGAGTGTTACGGGTGGCAGCGGTCCATTCCGTCCGGTCCGGAGCGGGAGG 2280
 C D K T H T C P C P

 A M
L N
U L
1 1

 B
B N SM F
A L PA O
N A 1E K
1 4 21 1

 BS S
SC F
TR A
NF N
11 1

 S
S
DHNA
RALU
AEA9
2346

 S
HNC
PCR
AIF
211

2281 AGCTCAAGGCGGGACAGGTGCCCTAGAGTAGCCTGCATCCAGGGACAGGCCCCAGCCGGG
 TCGAGTTCGCCCTGTCCACGGGATCTCATCGGACGTAGGTCCTGTCCGGGGTCGGCCC 2340

 A M M
F A B
L E O
3 2 2

 M D
N D
L E
1 1

 M
N
L
1

 BS S
SC M ANA M
TR B VLU B
NF O AA9 O
11 2 246 2

2341 TGCTGACACGTCCACCTCCATCTCTTCTCAGCACCTGAACTCCTGGGGGGACCGTCAGT
 ACGACTGTGCAGGTGGAGGTAGAGAAGGAGTCGTGGACTTGAGGACCCCCCTGGCAGTCA 2400

A P E L L O G P S V -

-34-

M	S
N	T
L	Y
1	1

S	SS
AN	M HMANNAC DM M
UL	N PNYCLUR DS A
SA	L ALAIA9F ET E
A3	1 2121461 12 3

2401 CTTCCTCTTCCCCCAAACCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCAC 2460
 GAAGGAGAAGGGGGGTTTTGGGTTCTGTGGGAGTACTAGAGGGCCTGGGACTCCAGTG

F L F P P K P K D T L M I S R T P E V T -

N							
NS	M	M	DM	M		RM	M
LP	A		DS	B		SA	N
AH	E	L	ET	O		AE	L
31	2	1	12	2		12	1

2461 ATGCGTGGTGGTGGACGTGAGCCACGAAGACCCCTGAGGTCAAGTTCAACTGGTACGTGGA 2520
 TACGCACCACCACCTGCACTCGGTGCTTCTGGGACTCCAGTTCAAGTTGACCATGCACCT

C V V V D V S H E D P E V K F N W Y V D -

	F FN		
M	N NSS	R	M R
N	U UPA	S	A S
L	4 DBC	A	E A
1	H 222	1	2 1

2521 CGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGGGGAGGAGCAGTACAACAGCACGTA 2580
 GCGGCACCTCCACGTATTACGGTTCTGTTTCGGCGCCCTCCTCGTCATGTTGTCTGTCAT

G V E V H N A K T K P R E E Q Y N S T Y -

S		BS	
HNC MH	M	SC	R
PCR GP	N	TR	S
AIF AH	L	NF	A
211 11	1	11	1

2581 CCGGGTGGTCAGCGTCTCACCCTCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAA 2640
 GGGCCACCAGTCGAGGAGTGGCAGGACGTGGTCTGACCGACTTACCGTTCTCATGTT

R V V S V L T V L H Q D W L N G K E Y K -

M	T
N	A
L	Q
1	1

2641 GTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACCATCTCCAAGCCAA 2700
 CACGTTCCAGAGGTTGTTTCGGGAGGGTCCGGGGTAGCTCTTTTGCTAGAGGTTTCGGTT
 C K V S N K A L P A P I E K T I S K A K -

-35-

P S
ADNPMIA
VRLUNU
AAAAML9
2244116

S
A H M N
U A N L
9 E L A
6 3 1 3

S
HNN BSAH
APA GFUA
EAE LI9E
321 1163

//// /

2701 AGGTGGGACCCGTGGGGTGGGAGGGCCACATGGACAGAGGCCGGCTCGGCCACCCCTCTG
TCCACCCTGGGCACCCACGCTCCCGGTGTACCTGTCTCCGCCGAGCCGGGTGGGAGAC 2760

D M M
D N A
E L E
1 1 3

N S R
P S A
B A
2 1

M N L
1

F N A
U V A
H 1

B B V
1

2781 CCCTGAGAGTGACCGCTGTACCAACCTCTGTCTACAGGGCAGCCCCGAGAACCACAGGT
CGGACTCTCACTGGCGACATGGTTGGAGACAGGATGTCCCGTCGGGGCTCTTGCTGTCCA 2820

G Q P R E P Q V -

R F
S O
A K
1 1

SS
AHNCCS
VPCCRRM
AAIIFFA
1211111

A L U
1

F D K
1

BS
SC TR
NF 11

BS
SC TR
NF 11

2821 GTACACCCTGCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCT
CATGTGGGACGGGGTAGGGCCCTACTCGACTGGTTCTTGCTCCAGTCGGACTGGACGCA 2880

Y T L P P S R D E L T K N Q V S L T C L -

B
S
P
M
1

F
N H
U P
4 A
H 2

2881 GGTCAAAGGCTTCTATCCCAGCGACATCCCGTGGAGTGGGAGAGCAATGGGCAGCCGGA
CCAGTTTCCGAAGATAGGGTGGCTGTAGCGGCACCTCACCTCTCGTTACCCGTGGGCT 2940

V K G F Y P S D I A V E W E S N G Q P E -

B
B
V
1

H
M I
N N
L F
1 1

M B O
2

N L A
4

H
P
H
1

2941 GAACAACCTACAAGACCACGCTCCCGTGGTGGACTCCGACGGCTCCTTCTTCTCTACAG
CTTGTTGATGTTCTGGTGGCAGGGCAGCACCTGAGGCTGCCGAGCAAGAAGGAGATGTC 3000

N N Y K T T P P V L D S D G S F F L Y S -

-36-

M A	B	F		S
N L	S	NM	MEX	NF M
L U	P	UB	ABM	LA N
1 1	M	40	EVN	AN L
	1	H2	211	31 1

3001 CAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGAT / 3060
 GTTCGAGTGGCACCTGTTCTCGTCCACCGTCGTCCCCTTGCAGAAGACTACGAGGCACTA
 K L T V D K S R W Q Q G N V F S C S V M -

N N			S
S L		M M	MNC
I A		B N	PCR
1 3		O L	AIF
		2 1	211

3061 GCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATG / 3120
 CGTACTCCGAGACGTGTTGGTGATGTGCGTCTTCTCGAGAGGGACAGAGGCCCATTTAC
 H E A L H N H Y T Q K S L S L S P G K .

CXHN
 FMAPA
 RAEAE
 13321
 /
 3121 AGTCCGACGGCCG 3133
 TCACGCTGCCGCC

-37-

Table 2

F N				S	
N S	B	M	H	DNA	B
U P	B	N	G	RAU	S
4 B	V	L	A	AE9	T
H 2	1	1	1	238	X

GCCTGTTTGAGAAGCAGCGGGCAAGAAAGACGCAAGCCAGAGGCCCTGCCATTTCTGTG
 1 ----- 60
 CGGACAAACTCTTCGTGCGCCGTTCTTTCTGCGTTCGGGTCTCCGGGACGGTAAAGACAC

B	PS		S		S	
DBS	ADNPA	D	DHNA	M	HM	HNC
DAP	VRLUU	D	RALU	N	AN	PCR
EN1	AAAM9	E	AEA9	L	EL	AIF
122	22416	1	2346	1	31	211

GGCTCAGGTCCCTACTGGCTCAGGCCCTGCCTCCCTCGGCAAGGCCACAATGAACCGGG
 61 ----- 120
 CCGAGTCCAGGGATGACCGAGTCCGGGACGGAGGGAGCGTTCCGGTGTACTTGGCCC

M N R G -

H		F		F	
I	B	N	HM	NM	D
N	B	U	HA	UN	D
F	V	4	AE	4L	E
1	1	H	12	H 1	1

121 GAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGTCTCTCCAGCAGCCACTC
 ----- 180
 CTCAGGCAAAATCCGTGAACGAAGACCACGACGTTGACCGCGAGGAGGGTCTGCGGTGAG

V P F R H L L L V L Q L A L L P A A T Q -

B	E	E		R	A
B	C	C		S	L
V	O	O		A	U
1	K	K		1	1

181 AGGGAAAGAAAGTGCTGCTGGGCAAAAAAGGGGATACAGTGGAAGTACCTGTACAGCTT
 ----- 240
 TCCCTTTCTTTACCACGACCCGTTTTTCCCTATGTCACCTTGACTGGACATGTCGAA

G K K V V L G K K G D T V E L T C T A S -

	M	M		H
	B	B		I
	O	O		N
	2	2		F

241 CCCAGAAGAAGAGCATACAATTCCTGCAAAAACTCCAACCAGATAAAGATTCTGGGAA
 ----- 300
 GGGTCTTCTTCTCGTATGTTAAGGTGACCTTTTTGAGGTTGGTCTATTTCTAAGACCCTT

Q K K S I Q F H W K N S N Q I K I L G N -

-38-

B		S		S	F	H
NBS	F	AA	A	A	NH	I
LAP	O	VU	L	U	UH	N
AN1	K	A9	U	3	DA	F
422	1	26	1	A	21	1

301 ATCAGGGCTCCTTCTTAAGTAAAGGTCCATCCAAGCTGAATGATCGCGCTGACTCAAGAA
 TAGTCCCGAGGAAGAATTGATTTCAGGTAGGTTGACTTACTAGCGCGACTGAGTTCTT 360
 Q G S F L T K G P S K L N D R A D S R R -

S		S		H		H
MANAS		BA	I	A		ID
BVLUT		CU	N	F		ND
OAA9Y		L3	F	L		FE
22461		1A	1	2		11

361 GAAGCCTTTGGGACCAAGGAAACTTCCCCCTGATCATCAAGAATCTTAAGATAGAAGACT
 CTTGGAAACCTGGTTCCTTTGAAGGGGACTAGTAGTTCTTAGAATTCTATCTTCTGA 420
 S L W D Q G N F P L I I K N L K I E D S -

M	M	S		M
B	N	AMAM		A
O	L	VNUN		E
2	1	AL9L		1
		2161		

421 CAGATACTTACATCTGTGAAGTGGAGGACCAGAAGGAGGAGGTGCAATTGCTAGTGTTCG
 GTCTATGAATGTAGACACTTCACCTCCTGGTCTTCTCCTCCACGTTAACCATCACAAGC 480
 D T Y I C E V E D Q K E E V Q L L V F G -

		B		S
		S		T
		P		Y
		M		1
		1		

481 GATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCGAGAGCCTGACCCCTGACCTTGG
 CTAAGTACGCGTTGAGACTGTGGGTGGACGAAGTCCCCGTCTCGGACTGGGACTGGAACC 540
 L T A N S D T H L L Q G Q S L T L T L E -

B	BS			H		S
BS	SC	D	M	I	N	T
AP	TR	E	N	F	Y	
N1	NF	1	1	1	1	
22	11					

541 AGAGCCCCCTGGTAGTACCCCTCAGTGCAATGTAGGAGTCCAAGGGGTAAAAACATAC
 TCTCGGGGGGACCATCATCGGGGAGTCAGTTACATCCTCAGGTTCCCCATTTTGTATG 600
 S P P G S S P S V Q C R S P R G K N I Q -

-39-

			N	BBH S	B	BS
M	MD	ASP	A	BSSGSC	S	BN SC
B	ND	LPV	L	APTIAR	T	AL TR
O	LE	UBU	U	N1NACF	X	NA NF
2	11	122	1	221111	1	1 4 11

601 AGGGGGGAAGACCCCTCTCCGTGTCTCAGCTGGAGCTCCAGGATAGTGGCACCTGGACAT
 TCCCCCCTTCTGGGAGAGGCACAGAGCTCGACCTCGAGGTCTATCACCGTGGACCTGTA 660
 G G K T L S V S Q L E L Q D S G T W T C -

N			
NS			
LP		M	
AH		B	
31		O	
/		2	
			NM A
			HA L
			EE U
			11 1

661 GCACTGTCTTGCAGAACCAGAAGAAGGTGGAGTTCAAAATAGACATCGTGGTCTAGCTT
 CGTGACAGAACGTCTTGGTCTTCTCCACCTCAAGTTTTATCTGTAGCACCAGCATCGAA 720
 T V L Q N Q K K V E F K I D I V V L A F -

HS	M	M
AT	N	N
EU	L	L
31	1	1

721 TCCAGAAGGCCTCCAGCATAGTCTATAAGAAAGAGGGGGAACAGGTGGAGTCTCCTTCC
 AGGTCTTCCGGAGGTCTATCAGATATTCTTCTCCCCCTTGTCCACCTCAAGAGGAAGG 780
 Q K A S S I V Y K K E G E R V E F S F P -

	A		A	M
	L		L	N
	U		U	L
	1		1	1

781 CACTCGCCTTTACAGTTGAAAAGCTGACGGGCGAGTGGCGAGCTGTGGTGGCAGCGGAGA
 GTGAGCGGAAATGTCAACTTTTCTGACTGCCCCGTCCCGCTCGACACCACCGTCCGCTCT 840
 L A F T V E K L T G S G E L W W Q A E R -

	P	S		
H	M	FM	A	
P	N	LN	U	M
H	L	ML	3	B
1	1	11	A	2

841 GGGCTTCTCCTCCAAGTCTTGGATCACCTTTGACCTGAAGAACAAGG AGTGTCTGTAA
 CCCGAAGGAGGAGGTTCAGAACCTAGTGGAACTGGACTTCTTGTCTCTCACAGACATT 900
 A S S S K S W I T F D L K N K E V S V K -

-40-

B	BS	PS			
SM	SCADNPAD	A			A H
TA	TRVRLUUD	L			L P
EE	NFAAAM9E	U			U H
23	11224161	1			1 1

901 AACGGGTTACCCAGGACCCTAAGCTCCAGATGGGCAAGAAGCTCCCGCTCCACCTCACCC 960
 TTGCCCAATGGGTCTCTGGGATTGAGGTCTACCCGTTCTTCGAGGGCGAGGTGGAGTGGG
 R V T Q D P K L Q M G K K L P L H L T L -

BS					BSS
M SC HS	D	M H			SCAHM
N TR AT	D	N P			TRUAN
L NF EU	E	L H			NF9EL
1 11 31	1	1 1			11631

961 TGCCCCAGGCCTTGCCCTCAGTATGCTGGCTCTGAAACCTCACCTGGCCCTTGAAGCGA 1020
 ACGGGGTCCGGAACGGAGTCATACGACCGAGACCTTTGGAGTGGGACCGGGAACCTTCGCT
 P Q A L P Q Y A G S G N L T L A L E A K -

S	BS				
F	SC				H D A
A	TR				P D L
N	NF				H E U
1	11				1 1 1

1021 AAACAGGAAAGTTGCATCAGGAAGTGAACCTGGTGGTGATGAGAGCCACTCAGTCCAGA 1080
 TTTGTCCTTCAACGTAGTCCTTCACTTGGACCACCACTACTCTCGGTGAGTCGAGGTCT
 T G K L H Q E V N L V V M R A T Q L Q K -

	PS	S			
M	ADNPA	DF	AM	DE	A
N	VRLLUJ	DA	LN	DS	L
L	AAAAM9	EN	UL	EP	U
1	224416	11	11	11	1

1081 AAAATTTGACCTGTGAGGTGTGGGGACCCACCTCCCTAAGCTGATGCTGAGCTTGAAC 1140
 TTTTAAACTGGACACTCCACACCCCTGGGTGGAGGGGATTGCACTACGACTCGAAGCTTG
 N L T C E V W G P T S P K L M L S L K L -

M	T	H	M	DM
N	A	P	N	DS
L	Q	A	L	ET
1	1	2	1	12

1141 TGGAGAACAAGGAGGCAAGGTCTCGAAGCGGCGAGAAGCCGGTGTGGGTGCTGAACCTG 1200
 ACCTCTTGTTCCTCCGTTTCCAGAGCTTCGCCCTCTTCGGCCACACCCACGACTTGGGAC
 E N K E A K V S K R E K P V W V L N P E -

-41-

				H		PS		H
F	D	M	I	A		ADPA		I
O	D	A	N	V		VRUJ		N
K	E	E	F	A		AAM9		F
1	1	3	1	1		2214		1

///

1201 AGCGCGGGATGTGGCAGTGTCTGCTGAGTGACTCGGCACAGGTCTGCTGGAATCCAACA
 TCCGCCCTACACCGTCACAGACGACTCACTGAGCCCTGTCCAGGACGACCTTAGGTTGT 1260

A G M W Q C L L S D S G Q V L L E S N I -

S	SA	BHF BS	H
ANA	HNCB	SGHMAANXA	RSD I A
VLU	PCRA	PIUNMULHV	SCD N L
AA9	AIFL	1ADLH3A0A	AAE D U
236	2111	21211A421	111 3 1

1261 TCAAGGTTCTGCCACATGGTCCACCCCGGTGCACGCGGATCCCGAGGGTGAGTACTAAG
 AGTTCCAAGACGGGTGTACCAGGTGGGGCCACGTGCGCCTAGGCGTCCCACTCATGATTC 1320

K V L P T W S T P V H A D P E

	E	BS	SS	F	BS	F
H	CHH	SC	HNCB	N	BSC	N
P	OHA	TR	PGCRA	U	BTR	U
H	4AE	NF	AAIFN	4	VNF	4
1	712	1	21111	H	111	H

1321 CTTCAGCGCTCCTGCCTGGACGCATCCCGGCTATGCAGCCCCAGTCCAGGGCAGCAAGGC
 GAAGTCGGGAGGACGGACCTGCGTAGGGCCGATACGTGGGGGTGAGTCCCGTCTCCG 1380

S	S	M	MNDM
DBMVMNA	HVMCN	N	NLDB
RB4BPLU	PNCRL	L	LAEO
AVEOHA9	ALIFA	1	1312
2132146	21114		

1381 AGGCCCCGTCTGCTCTTCCACCGGAGCCTCTGCCCCCCCCACTCATGCTCAGGGAGAGG
 TCCGGGCGAGACGGAGAAGTGGGCCTCGGAGACGGGGGGGTGAGTACGAGTCCCTCTCC 1440

BS	P	B	BS S
SC	F	M B N S	SCDHA
TR	L	A A L P	TRRAU
NF	M	E N A 1	NFAE9
11	1	1 1 4 2	11236

1441 GTCTTCTGGCTTTTTCCAGGCTCTGGGCAGGCACAGGCTAGGTGCCCTAACCAGGCG
 CAGAAGACCGAAAAAGGGTCCGAGACCGTCCGTGTCGATCCACGGGGATTGGGTCCG 1500

B S P M 1	B DBS DAP EN1 122	B S P M 1 1	S HNC PCR AIF 211	PS ADNPA VRLUJ AAAM9 22416
-----------------------	-------------------------------	----------------------------	-------------------------------	--

1501 CTGCACACAAAGGGGCGAGTCTGGGCTCAGACCTGCCAAGAGCCATATCCGGGAGGACC
 GACGTGTGTTTCCCGTCCACGACCCGAGTCTGGACGGTCTCGGTATAGCCCTCTGG 1560

D D E 1	H A E 3	D D E 1	A L U 1	M N L 1
------------------	------------------	------------------	------------------	------------------

1561 CTGCCCCCTGACCTAAGCCACCCCAAAGGCCAACTCTCCACTCCCTCAGCTCGGACACC
 GACGGGACTGGATTCTGGGTGGGGTTTCCGGTTTGAAGGTGAGGGAGTGGAGCCTGTGG 1620

H I N F 1	M N L 1	MM AB ED 32	P S T 1	B BS AP N1 22	M A E 3
-----------------------	------------------	----------------------	------------------	---------------------------	------------------

1621 TTCTCTCTCCAGATTCCAGTAACTCCCAATCTTCTCTGAGAGCCCAAATCTTGTG
 AAGAGAGGAGGGTCTAAGGTCATTGAGGGTTAGAAGAGAGAGCTCTCGGGTTTACAACAC 1680

E P K S C D -

N NS LP AH 3	BBS SSC PTR 1N= 211	BS SC HS TR AT NF EU 11 31	M A N L L U 1 1
--------------------------	---------------------------------	--	--------------------------

1681 ACAAACTCACACATGCCAGGCTGCCAGGTAAGCCAGCCAGGCTCGCCCTCCAGCT
 TGTTTTGAAGTGTGTACGGGTGGCAGGGTCCATTGGTGGGTCCGAGCGGGAGGTGCA 1740

K T H T C P P C P

M N L 1	B N A L N A 1 4	B SW F PA O 1E K 21 1	BS SC F TR A NF N 11 1	S DHNA RALU AEA9 2346	S HNC PCR AIF 211
------------------	--------------------------	-----------------------------------	------------------------------------	-----------------------------------	-------------------------------

1741 CAAGCGGGACAGGTGCCCTAGAGTAGCCTGCATCCAGGGACAGGCCAGCCGGGTGCT
 GTCCGCCCTGTCCACGGATCTCATCGGACGTAGGTCCCTGTCCGGGTGGGCCACGA 1800

-43-

A	M	M	M	D	M	BS	S
F	A	B	N	D	N	SC	M ANA M
L	E	0	L	E	L	TR	B VLU B
3	2	2	1	1	1	NF	0 AA9 0
						11	2 246 2

1801 GACACGTCCACCTCCATCTCTTCTCAGCACCTGAACCTCTGGGGGACCGTCAGTCTTC 1860
 CTGTGCAGGTGCAGGTAGAGAAGGAGTCTGTGGACTTGAGGACCCCCCTGGCAGTCAGAAG

A P E L L G G P S V F -

		S	SS	N
M	S	AN	M HMANNAC DM	NS
N	T	UL	N PMVCLUR DS	LP
L	Y	3A	L ALAIA9F ET	AH
1	1	A3	1 2121461 12	31

1861 CTCTTCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTACATGC 1920
 GAGAAGGGGGGTTTTGGGTTCCTGTGGGAGTACTAGAGGGCTGGGGACTCCAGTGTACC

L F P P K P K D T L M I S R T P E V T C -

M	M	DM	M	RM	M
A	N	DS	B	SA	N
E	L	ET	0	AE	L
2	1	12	2	12	1

1921 GTGGTGGTGGACGTGAGCCACGAAGACCTGAGGTCAAGTTCAACTGGTACGTGGACGGC 1980
 CACCACCACCTGCACTCGGTGCTTCTGGGACTCCAGTTCAAGTTGACCATGCACCTGCCG

V V V D V S H E D P E V K F N W Y V D G -

	F FN	S
N	N NSS	R
N	U UPA	S
L	4 DBC	A
1	H 222	1
		2
		1 211

1981 GTGGAGGTGCATAATGCCAAGACAAAGCCGGGGAGGAGCAGTACAACAGCAGTACCGG 2040
 CACCTCCACGTATTACGGTTCTGTTTCGGCGCCCTCCTCGTCATGTTGTCGTGCATGGCC

V E V H N A K T K P R E E Q Y N S T Y R -

-44-

HH		BS	
GP	M	SC	R
AH	N	TR	S
11	L	NF	A
	1	11	1

2041 GTGGTCAGCGTCCTCACCCTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGC 2100
 CACCAGTCGCAGGAGTGGCAGGACGTGGTCTGACCGACTTACCGTTCTCATGTTTCAGG

V V S V L T V L H Q D W L N G K E Y K C -

M	T
N	A
L	Q
1	1

2101 AAGGTCTCCAACAAAGCCCTCCAGCCCCATCGAGAAAACCATCTCCAAGCCAAAGGT 2160
 TTCCAGAGGTTGTTTCGGGAGGGTGGGGGGTAGCTCTTTTGGTAGAGGTTTCGGTTTCCA

K V S N K A L P A P I E K T I S K A K

P S	S		S	
ADNNPMA	A H M	N	HHN	BSAH
VRLLUNU	U A N	L	APA	GFLA
AAAAML9	9 E L	A	EAE	LI9E
2244116	6 3 1	3	321	1163
				1

2161 GGGACCCGTGGGGTGGCAGGGCCACATGGACAGAGGCCGGCTCGGCCACCCCTCTGCCCT 2220
 CCCTGGGCACCCACGCTCCCGGTGTACCTGTCTCCGGCCGAGCCGGTGGCAGACGGGA

M	M	N		M	F	A	B	R F
N	A	P S		N	U	V	B	S O
L	E	B A		L	4	A	V	A K
1	3	2 1		1	H	1	1	1 1

2221 GAGAGTGACCGCTGTACCAACCTCTGTCTACAGGGCAGCCCCGAGAACCACAGGTGTAC 2280
 CTCTCACTGGCGACATGGTTGGAGACAGGATGTCCCGTGGGGCTCTTGGTGTCCACATG

G Q P R E P Q V Y -

-45-

SS			BS		BS B
AMVNCCS	A	F	SC		SC S
VPCCPRV	L	O	TR		TR P
AAIIFFA	U	K	NF		NF M
1211111	1	1	11		11 1

2281 ACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTC
 TGGGACGGGGGTAGGGCCCTACTCGACTGGTTCTTGGTCCAGTCGGACTGCACGGACCAG 2340
 T L P P S R D E L T K N Q V S L T C L V -

2341 AAAGGCTTCTATCCACCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGACAAC
 TTTCCGAAGATAGGGTCGCTGTAGCGGCACCTCACCTCTCGTTACCCGTGGCCTCTTG 2400
 K G F Y P S D I A V E W E S N G Q P E N -

2401 AACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAG
 TTGATGTTCTGGTGGGAGGGCAGGACCTCAGGCTGCCGAGGAAGAAGGAGATGTCGTTG 2460
 N Y K T T P P V L D S D G S F F L Y S K -

2461 CTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCAT
 GAGTGGCACCTGTTCTCGTCCACCGTCGTCCCTTGCAGAAGAGTACGAGGCACTACGTA 2520
 L T V D K S R W Q Q C N V F S C S V M H -

N
L
A
3

		S
M	M	HNC
B	N	PCR
0	L	AIF
2	1	211

2521 GAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGTAAATGAGTG

CTCCGAGACGTGTTGGTGATGTCCGTCTTCTCGGAGACGGACAGAGGCCCATTTACTCAC 2580

E A L H N H Y T Q K S L S L S P G K .

CXH
FMA
RAE
133

2581 CGACGGCCG
----- 2589
GCTGCCGGC

V W
 B B
 C C
 2 2
 W I N F
 1
 CCCAGAAGAAGAGCATACAATTCCACTGGAAAACTCCAACCAGATAAAGATTCTGGGAA
 241 ----- 300
 GGGTCTTCTTCTCGTATGTTAAGGTGACCTTTTGGAGCTTGGTCTATTCTAAGACCCTT
 Q K K S I Q F H W K N S N Q I K I L G N -
 B S
 NBS AA
 LAP O VU
 AN1 K A9
 422 1 26
 S A S F H
 A U N H I
 3 U U H N
 A 2 1 1
 ATCAGGGCTCCTTCTTAAGTAAAGGTCCATCCAAGCTGAATGATCGCGCTGACTCAAGAA
 301 ----- 360
 TAGTCCCAGGAAGAATTGATTTCCAGGTAGGTCGACTTACTAGCGCGACTGAGTTCTT
 Q G S F L T K G P S K L N D R A D S R R -
 S
 MANAS
 BVLUT
 OAA9Y
 22461
 S BA H A H
 CU N I F I D
 L3 F L F E
 1A 1 2 1 1
 GAAGCCTTTGGGACCAAGGAAACTTCCCCCTGATCATCAAGAATCTTAAGATAGAAGACT
 361 ----- 420
 CTTCCGAAACCCTGGTTCCTTTGAAGGGGGACTAGTAGTTCTTAGAATTCTATCTTCTGA
 S L W D Q G N F P L I I K N L K I E D S -
 S
 M M AMAM
 B N VNUN
 O L AL9L
 2 1 2161
 M A E
 1
 CAGATACTTACATCTGTGAAGTGGAGGACCAGAAGGAGGAGGTGCAATTGCTAGTGTTCG
 421 ----- 480
 GTCTATGAATGTAGACACTTCACCTCCTGCTTCTCCTCCACGTTAACGATCACAAGC
 D T Y I C E V E D Q K E E V Q L L V F G -

-50-

HS	M	M
AT	N	N
EU	L	L
31	1	1

TCCAGAAGGCCTCCAGCATAGTCTATAAGAAAGAGGGGAACAGGTGCAGTTCTCCTTCC
 721 ----- 780
 AGGTCTTCGGGAGGTGCTATCAGATATTCTTTCTCCCCCTTGTCACCTCAAGAGGAAGG
 Q K A S S I V Y K K E G E Q V E F S F P -

	A	A	M
	L	L	N
	U	U	L
	1	1	1

CACTCGCCTTTACAGTTGAAAAGCTGACGGGCAGTGGCGAGCTGTGGTGGCAGGCGGAGA
 781 ----- 840
 GTGAGCGGAAATGTCAACTTTTCGACTGCCCGTCAACGCTCGAACCACCGTCCGCCTCT
 L A F T V E K L T G S G E L N W Q A E R -

	P	S	
H	M	FV	A
P	N	LN	U
H	L	ML	3
1	1	11	A
			2

GGGCTTCCTCCTCCAAGTCTTGATCACCTTTGACCTGAAGAACAAGGAAGTGTCTGTAA
 841 ----- 900
 CCCGAAGGAGGAGGTTCAAACTAGTGGAACTGGACTTCTTGTCTTCACAGACATT
 A S S S K S W I T F D L K N K E V S V K -

B	BS	PS	
SM	SCADNPAD	A	AH
TA	TRVRLUUD	L	LP
EE	NFAAAM9E	U	UH
23	11224161	1	11

AACGGGTTACCCAGGACCCTAAGCTCCAGATGGGCAAGAAGCTCCCGTCCACCTCACCC
 901 ----- 960
 TTGCCCAATGGGTCCTGGGATTGAGGTCTACCCGTTCTTCGAGGGCGAGGTGGAGTGGG
 R V T Q D P K L Q M G K K L P L H L T L -

BS		BSS
M SC HS	D	SCAHM
N TR AT	D	TRUAN
L NF EU	E	NF9EL
1 11 31	1	11631

TCCCCCAGGCCTTGCTCAGTATGCTGGCTCTGGAACCTCACCTGGCCCTTGAAGCGA
 961 ----- 1020
 ACGGGGTCCGGAACGGAGTCATACGACCGAGACCTTTGAGTGGGACCGGGAACCTTCGCT
 P Q A L P Q Y A G S G N L T L A L E A K -

-51-

S	BS		
F	SC	HD	A
A	TR	PD	L
N	NF	HE	U
1	11	11	1

1021 AAACAGGAAAGTTGCATCAGGAAGTGAACCTGGTGGTGATGAGAGCCACTCAGCTCCAGA 1080
TTTGTCTTTCAACGTAGTCCTTCACTTGGACCACCACTACTCTCGGTGAGTCGAGGTCT
T G K L H Q E V N L V V M R A T Q L Q K -

	PS	S		
M	ADNPA	DF	AM	DE
N	VRLLUJ	DA	LN	DS
L	AAAAV9	EN	UL	EP
1	224415	11	11	11

1081 AAAATTTGACCTGTGAGGTGTGGGGACCCACCTCCCTTAAGCTGATGCTGAGCTTGAAC 1140
TTTTAACTGGACACTCCACACCCCTGGCTGGAGGGGATTGACTACGACTCGAACTTTG
N L T C E V W G P T S P K L M L S L K L -

M	T	H	M	DM
N	A	P	N	DS
L	Q	A	L	ET
1	1	2	1	12

1141 TGGAGAACAAGGAGGCAAAGGTCTCGAAGCGGGAGAAGCCGGTGTGGGTGCTGAACCCCTG 1200
ACCTCTTGTTCTCCGTTTCCAGAGCTTCGCCCTCTTCGGCCACACCCACGACTTGGGAC
E N K E A K V S K R E K P V W V L N P E -

	H	PS	H
F	DMIA	ADPA	I
O	DANV	VRUJ	N
K	EEFA	AAV9	F
1	11311	2216	1

1201 AGGCGGGGATGTGCCAGTGTCTGCTGAGTCACTCGGCACAGGTCCTGCTGGAATCCAACA 1260
TCCGCCCCCTACACCGTCACAGACGACTCACTGAGCCCTGTCCAGGACGACCTTAGGTTGT
A G M W Q C L L S D S G Q V L L E S N I -

-52-

	S	SA	BMF BS		H
	ANA	HMCN	SCNKAANDXA		RSD I A
	VLU	PCRA	PIUNMULHY		SCD N L
	AA9	AIFL	1ADLH3ADA		AAE D U
	236	2111	21211A421		111 3 1
	//	//	//		//
1261	TCAAGGTTCTGCCCACATGGTCCACCCCGGTGCACGCGGATCCCGAGGGTGAGTACTAAG				
	AGTTCCAAGACGGGTGTACCAGGTGGGGCCACGTGCGCTAGGGCTCCCACTCATGATTC				
	K V L P T W S T P V H A D P E				

	E	BS	SS	F	BS	F
H	CHH	F	SC	HMCN	N	BSC
P	OMA	O	TR	PGCRA	U	BTR
H	4AE	K	NF	AAIFN	4	VNF
1	712	1	11	21111	H	111
	/	/	/	/	/	/
1321	CTTCAGCGCTCCTGCCTGGACGCATCCCGGCTATGCAGCCCCAGTCCAGGGCAGCAAGGC					
	GAAGTCGGCAGGACGGACCTCGGTAGGGCCGATACGTGGGGTCAGGTCCCGTCGTTCCG					

	S	S		
	DBHMA	HMCN		VNDV
	RBABPLU	PNCRL		NDB
	AVEOMA9	ALIFA		LAEO
	2132146	21114		1312
	//	//		//
1381	AGGCCCCGTCTGCCTCTTCACCCGGAGCCTCTGCCCGCCCCACTCATGCTCAGGGAGAGG			
	TCCGGGGCAGACGGAGAAGTGGGCCTCGGAGACGGCGGGGTGAGTACGAGTCCCTCTCC			

	BS	P		B	BS	S
	SC	F		W	SCDMA	
	TR	L		A	TRRAU	
	NF	M		E	NFAE9	
	11	1		1	11236	
	/	/		/	/	
1441	GTCTTCTGGCTTTTTCCAGGCTCTGGGCAGGCACAGGCTAGGTGCCCCCTAACCCAGGCC					
	CAGAAGACCGAAAAAGGTCGAGACCCGTCCGTGTCCGATCCACGGGGATTGGGTCCGG					

	B	B	B	S	PS
	S	DBS	S	HNC	ADNPA
	P	DAP	P	PCR	VRLUJ
	M	EN1	M	AIF	AAAM9
	1	122	1	211	22416
	/	/	/	/	/
1501	CTGCACACAAAGGGGCAGGTGCTGGGCTCAGACCTGCCAAGACCATATCCGGGAGGACC				
	GACGTGTGTTTCCCCGTCCAGACCCGAGTCTGGACGGTTCTCGGTATAGGCCCTCTGG				

-53-

D		H		D	A	M
D		A		D	L	N
E		E		E	U	L
1		3		1	1	1

1561 CTGCCCCGACCTAAGCCACCCCAAAGGCCAACTCTCCACTCCCTCAGCTCGGACACC

 GACGGGGACTGGATTGGGTGGGGTTTCCGGTTTCAGAGGTGAGGGAGTCGAGCCTGTGG 1620

L P L T * A H P K G Q T L H S L S S D T -
 C P * P K P T P K A K L S T P S A R T P -
 A P D L S P P Q R P N S P L P Q L G H L -

H				DF		S
I	M	MM		DO		F
N	N	AB		EK		A
F	L	EO		11		N
1	1	32				1

1621 TTCTCTCTCCAGATTCCAGTAACTCCCAATCTTCTCTCTCAGGGAGTGCATCCGCCCC

 AAGAGAGGAGGGTCTAAGGTCATTGAGGGTTAGAAGAGAGAGTCCCTCACGTAGCGGGG 1680

G S A S A P -

				E
	V			C
	N			O
	L			R
	1			1

1681 AACCCCTTTTCCCCCTCGTCTCTGTGAGAATTCC....

 TTGGGAAAAGGGGGAGCAGAGGACACTCTTAAGC.... 1714
 T L F P L V S C E N S

-55-

M M M
B B I
O O N
F
2 2 1
241 CCCAGAAGAAGAGGCATACAATTCCACTGGAAAACTCCAACCASATAAAGATTCTGGGAA

GGGTCTTCTTCTCGTATGTTAAGGTGACCTTTTTGAGGTTGGTCTATTCTAAGACCCCT 300

Q K K S I Q F H W K N S N Q I K I L G N -

	B		S		S	F	H
	NBS	F	AA	A	A	N	I
	LAP	O	VU	L	U	U	N
	AN1	K	A9	U	S	D	F
	422	1	26	1	A	2	1
	/		/				
301	ATCAGGGCTCCTTCTTAATAAGGTCCATCCAAGCTGAATGATCGCGCTGACTCAAGAA						

	TAGTCCCGAGGAAGAATTGATTTCCAGGTAGGTTGACTTACTAGCGCGACTGAGTTCTT						
	360						

Q G S F L T K G P S K L N D R A D S R R -

	S		S		H		H	
	MANAS		BA		I	A	I	D
	BVLUT		CU		N	F	N	D
	QAA9Y		L3		F	L	F	E
	22461		1A		1	2	1	1
	/		/					
361	GAAGCCTTTGGGACCAAGGAAACTTCCCCCTGATCATCAAGAATCTTAAGATAGAAGACT							

	CTTCGGAAACCCTGGTTCCTTTGAAGGGGGACTAGTAGTTCCTTAGAATTCTATCTTCTGA							

								420

S L W D Q G N F P L I I K N L K I E D S -

			S	
M	M		AMAM	
B	N		VNUN	M
O	L		ALOL	A
2	1		2161	E
			//	1

421 CAGATACTTACATCTGTGAAGTGGAGGACCAGAAGGAGGAGGTGCAATTGCTAGTGTTCG

 GTCTATGAATGTAGACACTTCACCTCCTGGTCTTCCTCCTCCAGTTAACGATCACAAGC 480

D T Y I C E V E D Q K E E V Q L L V F G -

-56-

B
S
P
M
1

S
T
Y
1

481 GATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGAGAGCCTGACCCCTGACCTTGG

CTAACTGACGGTTGAGACTGTGGGTGGACGAAGTCCCCGTCTCGGACTGGGACTGGAACC 540
L T A N S D T H L L Q G Q S L T L T L E -

B	BS			H	
BS	SC		D	M	I
AP	TR		D	N	S
N1	NF		E	L	T
22	11		1	1	1

541 AGAGCCCCCTGGTAGTAGCCCCCTCAGTGCAATGTAGGAGTCCAAGGGGTAAAAACATAC

TCTCGGGGGGACCATCATCGGGGAGTCACGTTACATCCTCAGGTTCCCCATTTTGTATG 600
S P P G S S P S V Q C R S P R G K N I Q -

		N	BBH S	B	BS
M	MD	ASP	A BSSGSC	S	BN
B	ND	LPV	L APTIAR	T	AL
O	LE	UBU	U N1NACF	X	NA
2	11	122	1 221111	1	1 4 11

601 AGGGGGGGAAGACCCTCTCCGTGTCTCAGCTGGAGCTCCAGGATAGTGGCACCTGGACAT

TCCCCCCTTCTCGGAGAGGCACAGAGTCGACCTCGAGGTCTATCACCCTGGACCTGTA 660
G G K T L S V S Q L E L Q D S G T W T C -

N			
NS		M	NM A
LP		B	MA L
AM		O	EE U
31		2	11 1

661 GCACTGTCTTGCAGAACCAGAAGAAGGTGGAGTTCAAAATAGACATCGTGGTGCTAGCTT

CGTGACAGAACGTCTTGGTCTTCTCCACCTCAAGTTTTATCTGTAGCACCAGGATCGAA 720
T V L Q N Q K K V E F K I D I V V L A F -

HS	M	M
AT	N	N
EU	L	L
31	1	1

721 TCCAGAAGGCCTCCAGCATAGTCTATAAGAAAGAGGGGGAACAGGTGCAGTTCTCCTTCC

AGGTCTTCCGAGGTCGTATCAGATATTCTTTCTCCCCCTGTCCACCTCAAGAGGAAGG 780
Q K A S S I V Y K K E G E Q V E F S F P -

-57-

A
L
U
1
A
L
U
1
M
N
L
1

CACTCGCCTTTACAGTTGAAAAGCTGACGGGCAGTGGCGAGCTGTGGTGGCAGGCGGAGA 840

 GTGAGCGGAAATGTCAACTTTTCGACTGCCCGTCACCGCTCGACACCACCGTCCGCTCT
 L A F T V E K L T G S G E L N N Q A E R -

P S
 H M F M A W
 P N L N U B
 H L M L S O
 1 1 1 1 A 2
 GGGCTTCCTCCTCCAAGTCTTGGATCACCTTTGACCTGAAGAACAAGGAAGTGTCTGTAA 900

 CCGGAAGGAGGAGGTT CAGAACCTAGTGGAAACTGGACTTCTTGTTCCTTCACAGACATT
 A S S S K S W I T F D L K N K E V S V K -

B	BS	PS		A	H
SM	SCADNPAD			L	P
TA	TRVRLUD			U	H
EE	NFAAAM9E			1	1
23	11224161				

/ / / /

AACGGGTTACCAGCACCTAAGCTCCAGATGGGCAAGAAGCTCCCGCTCCACCTCACCC 960

901 TTGCCAAATGGGTCTCTGGGATTTCAGGCTTACCCGTTCTTCGAGGGCGAGGTGGAGTG

R V T Q D P K L Q M G K K L P L H L T L -

	BS					BSS
M	SC	HS	D	M	H	SCAHM
N	TR	AT	D	N	P	TRUAN
L	NF	EU	E	L	H	NF9EL
1	11	31	1	1	1	11631

T G C C C A G G C C T T G C C T C A G T A T G C T G G C T C T G G A A A C C T C A C C C T G G C C C T T G A A G C G A
 961 ----- 1020
 A C G G G G T C C G G A A C G G A G T C A T A C G A C C G A G A C C T T G G A G T G G G A C C G G G A A C T T C G C T
 P Q A L P Q Y A G S G N L T L A L E A K -

	S	B	H	A
	F	S	D	
	A	C	P	L
	N	T	E	U
	1	11	11	1

/

AAACAGGAAAGTTGCATCAGGAAGTGAACCTGGTGGTGATGAGAGCCACTCAGCTCCAGA
1080

TTTGTCTTTCAACGTAGTCCTTCAC TTGGACCACCACTACTCTCGGTGAGTCCAGGTCT
1021

T G K L H Q E V N L V V M R A T Q L Q K -

-58-

M	PS	S			
N	ADNMPA	DF	AM	DE	A
L	VRLLUU	DA	LN	DS	L
1	AAAAW9	EN	UL	EP	U
	224416	11	11	11	1

1081 AAAATTTGACCTGTGAGGTGTGGGGACCCACCTCCCCTAAGCTGATGCTGAGCTTGAAC

 TTTTAACTGGACACTCCACACCCCTGGGTGGAGGGGATTGCACTACGACTCGAACTTTG 1140
 N L T C E V W G P T S P K L M L S L K L -

M	T	H	M	DM
N	A	P	N	DS
L	Q	A	L	ET
1	1	2	1	12

1141 TGGAGAACAAGGAGGCAAAGGTCTCGAAGCGGGAGAAGCCGGTGTGGGTGCTGAACCTG

 ACCTCTGTTCCTCCGTTTCCAGAGCTTCGCCCTCTTCGCCACACCCACGACTTGGGAC 1200
 E N K E A K V S K R E K P V W V L N P E -

	F	D	M	I	A	PS	H
	O	D	A	N	V	ADPA	I
	K	E	E	F	A	VRUU	N
	1	1	3	1	1	AAM9	F
						2216	1

1201 AGGCGGGGATGTGGCAGTGTCTGCTGAGTGAAGTCCCTGCTGGAATCCAACA

 TCCGCCCCCTACACCGTCACAGAGCACTCACTGAGCCCTGTCCAGGACGACCTTAGGTTGT 1260
 A G M W Q C L L S D S G Q V L L E S N I -

	S	SA	BHF	BS		H
	ANA	HNCP	SGNMA	AANXA		RSD I A
	VLU	PCRA	PIUNM	LHV		SCD N L
	AA9	AIFL	1ADLM	3A0A		AAE D U
	236	2111	21211	A421		111 3 1

1261 TCAAGGTTCTGCCACATGGTCCACCCCGGTGCACGGGATCCCGAGGCTGAGTACTAAG

 AGTTCCAAGACGGGTGTACCAGGTGGGGCCACGTGGCCCTAGGGCTCCCACTCATGATTC 1320
 K V L P T W S T P V H A D P E

	E	BS	SS	F	BS	F
H	CHH	F	SC	HNCF	N	BSC N
P	QHA	O	TR	PCCRA	U	BTR U
H	4AE	K	NF	AAIFN	4	VNF 4
1	712	1	11	21111	H	111 H

1321 CTTCAGCGCTCCTGCCTGGACGCATCCCGGCTATGCAGCCCCAGTCCAGGGCAGCAAGGC

 GAAGTCGCGAGGACGGACCTGCGTAGGGCCGATACGTGGGGTCAAGTCCCGTCTCCG 1390

S	S		
DBHM-HNA	HMNCN	M	MNDV
RBABPLU	PNCRL	N	NLDB
AVEOMA9	ALIFA	L	LAED
2132146	21114	1	1312
// //	//		

1381 AGGCCCCGTCTGCTCTTCCACCCGGAGCCTCTGCCCCCCCCACTCATGCTCAGGGAGAGG 1440

TCCGGGGCAGACGGAGAAGTGGGCTCGGAGACGGGCGGGGTGACTACGAGTCCCTCTCC

BS	P		B	BS	S
SC	F		M	SCDHA	
TR	L	M	B	TRRAU	
NF	M	A	N	NFAE9	
11	1	E	A	11236	
		1	1	4	2

1441 GTCTTCTGGCTTTTTTCCAGGCTCTGGGCAGGCACAGGCTAGGTGCCCCCTAACCCAGGCC 1500

CAGAAGACCGAAAAAGGTCGAGACCCGTCCTGTCCGATCCACGGGATTGGGTCCGG

B	B	B	S	PS
S	DBS	S	M	ADNPA
P	DAP	P	N	VRLUU
M	EN1	M	L	AAAM9
1	122	1	1	22416

1501 CTGCACACAAAGGGGCAGGTGCTGGGCTCAGACCTGCCAAGAGCCATATCCGGGAGGACC 1560

GACGTGTGTTTCCCGTCCACGACCCGAGTCTGGACGGTTCTCGGT/TAGGCCCTCTGG

D	H	D	A	M
D	A	D	L	N
E	E	E	U	L
1	3	1	1	1

1561 CTGCCCCCTGACCTAGCCCCACCCAAAGGCCAAACTCTCCACTCCCTCAGCTCGGACACC 1620

GACGGGCACTGGATTGGGTGGGGTTTCCGGTTTACAGGTGAGGGAGTCCAGCCTGTGG

H			F
I	M	MM	AN
N	N	AB	LU
F	L	EO	UA
1	1	32	1H

1621 TTCTCTCTCCAGATTCCAGTAACTCCCAATCTTCTCTCTGCAGTGATTGCTGAGCTGC 1680

AAGAGAGGAGGCTCTAAGGTCAATTGAGGGTTAGAAGAGAGACGTCACTAACGACTCGACC

V I A E L P -

M H M F
 B G N B U
 O A L O D
 2 1 1 2 2
 CTCCCAAGTGAGCGTCTTCGTCCCACCCCGCGACGGCTTCTTCGGCAACCCCCGCAAGT
 1681 ----- 1740
 GAGCGTTTCACTCCAGAACGAGGCTGGGGCGCTGCCGAAGAAGCCGTTGGGGCGTTCA
 P K V S V F V P P R D G F F G N P R K S -
 A BS S H B S F
 L S C H HNC I B SMC N
 U T R A PCR N B TNR U
 1 N F E AIF F V NLF 4
 11 3 211 1 1 111 H
 / / /
 CCAAGCTCATCTGCCAGGCCACGGGTTTCAGTCCCCGGCAGATTCAGGTGTCTGGCTGC
 1741 ----- 1800
 GGTTCGAGTAGACGGTCCGGTGCCCAAAGTCAGGGGCCGTCTAAGTCCACAGGACCGACG
 K L I C Q A T G F S P R Q I Q V S W L R -
 F B S BS H
 NH S H H AM AA SCM D H I
 LH P P G HA VU TRN D A N
 DA M H A AE A9 NFL E E F
 21 1 1 1 23 26 111 1 3 1
 / / /
 GCGAGGGGAAGCAGGTGGGGTCTGGCGTCACCACGGACAGGTCCAGGCTGAGGCCAAAG
 1801 ----- 1860
 CGCTCCCTTCTGTCCACCCAGACCGCAGTGGTGCTGGTCCAGTCCGACTCCGGTTTC
 E G K Q V G S G V T T D Q V Q A E A K E -
 SS B B
 AAHNABS SM H
 UUALPAP TA P
 09EAA1 EE H
 8634122 23 1
 / / /
 AGTCTGGGCCCCACGACCTACAAGGTGACCAGCACACTGACCATCAAAGAG....
 1861 ----- 1910
 TCAGACCCGGGTGCTGGATGTTCCACTGGTGGTGTGACTGGTAGTTTCTC....
 S G P T T Y K V T S T L T I K E

-61-

Table 5

	F N		B M		H S		B
	N S		B	M		DHA	S
	U P		B	N		RAU	T
	4 B		V	L		AE9	X
	H 2		1	1		236	1

1

GCCTGTTTGAGAAGCAGCGGGCAAGAAAGACGCAAGCCCAGAGGCCCTGCCATTTCGTG
----- 60
CGGACAAACTCTTCGTGCGCCGTTCTTTCTGCGTTCGGGTCTCCGGGACGGTAAAGACAC

B	PS		S				S
DBS	ADNPA	D	DHNA	M	HM		HNC
DAP	VRLUU	D	RALU	N	AN		PCR
EN1	AAAM9	E	AEA9	L	EL		AIF
122	22416	1	2346	1	31		211

61

GGCTCAGGTCCCTACTGGCTCAGGCCCTCGCTCCCTCGGCAAGGCCACAATGAACCGGG
----- 120
CCGAGTCCAGGGATGACCGAGTCCGGGACGGAGGGAGCCGTTCCGGTGTACTTGGCCC

M N R G -

H			F			F	
I		B	N		HH	N M	D
N		B	U		HA	U N	D
F		V	4		AE	4 L	E
1		1	H		12	H 1	1

121

GAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTCCTCCCAGCAGCCACTC
----- 180
CTCAGGGGAAATCCGTGAACGAAGACCACGACGTTGACCGCGAGGAGGGTCTGTCGGTGAG

V P F R H L L L V L Q L A L L P A A T Q -

B	E	E			R	A
B	C	C			S	L
V	O	O			A	U
1	K	K			1	1

181

AGGGAAGAAAGTGGTGCTGGGCAAAAAAGGGGATACAGTGGAAGTGCCTGTACAGCTT
----- 240
TCCCTTTCTTTCACCACGACCCGTTTTTCCCTATGTACCTTGACTGGACATGTGAA

G K K V V L G K K G D T V E L T C T A S -

						H
	M	M				I
	B	B				N
	O	O				F
	2	2				1

241

CCGAGAAGAAGAGCATACAATTCCACTGGAAAACTCCAACCAGATAAAGATTCTGGGAA
----- 300
GGGTCTTCTTCTCGTATGTTAAGGTGACCTTTTTGAGGTTGGTCTATTTCTAAGACCTT

Q K K S I Q F H W K N S N Q I K I L G N -

-62-

B		S		S	F	H
NBS	F	AA	A	A	NH	I
LAP	O	VU	L	U	UH	N
AN1	K	A9	U	3	DA	F
422	1	26	1	A	21	1

ATCAGGGCTCCTTCTTAAGTAAAGGTCCATCCAAGCTGAATGATCGCGCTGACTCAAGAA
----- 360
TAGTCCCGAGGAAGAATTGATTTCCAGGTAGGTTGACTTACTAGCGCGACTGAGTTCTT
Q G S F L T K G P S K L N D R A D S R R -

S		S		H		H
MANAS		BA		I	A	I D
BVLUT		CU		N	F	N D
OAA9Y		L3		F	L	F E
22461		1A		1	2	1 1

GAAGCCTTTGGGACCAAGGAACTTCCCCCTGATCATCAAGAATCTTAAGATAGAAGACT
----- 420
CTTCGGAAACCCTGGTTCCTTTGAAGGGGGACTAGTAGTCTTAGAATTCTATCTTCTGA
S L W D Q G N F P L I I K N L K I E D S -

		S		
M	M	AMAM		M
B	N	VNUN		A
O	L	AL9L		E
2	1	2161		1

CAGATACTTACATCTGTGAAGTGGAGGACCAGAAGGAGGAGGTGCAATTGCTAGTGTTGG
----- 480
GTCTATGAATGTAGACACTTCACCTCCTGGTCTTCTCCTCCACGTTAACGATCACAAGC
D T Y I C E V E D Q K E E V Q L L V F G -

		B		S
		S		T
		P		Y
		M		1
		1		

GATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTGACCCTGACCTTGG
----- 540
CTAACTGACGGTTGAGACTGTGGGTGGACGAAGTCCCCGTCTGGACTGGGACTGGAACC
L T A N S D T H L L Q G Q S L T L T L E -

-63-

B BS
BS SC
AP TR
N1 NF
22 11

D
D
E
1

M
N
L
1

H
I
N
F
1
S
T
Y
1

AGAGCGCCCTGGTAGTAGCCCTCAGTGCAATGTAGGAGTCCAAGGGGTAAAAACATAC 600
541 TCTCGGGGGGACCATCATCGGGGAGTCACGTTACATCCTCAGGTTCCCATTTTGTATG
S P P G S S P S V Q C R S P R G K N I Q -

		N	BBH S	B	BS
M	MD	ASP	A BSSGSC	S	B N SC
B	ND	LPV	L APTIAR	T	A L TR
O	LE	UBU	U N1NACF	X	N A NF
2	11	122	1 221111	1	1 4 11

AGGGGGGGAAGACCCTCTCCGTGTCTCAGCTGGAGCTCCAGGATAGTGGCACCTGGACAT 660
601 TCCCCCCTTCTGGGAGAGGCACAGAGTCGACCTCGAGGTCCTATCACCCTGGACCTGTA
G G K T L S V S Q L E L Q D S G T W T C -

N		M	NM A
NS		B	HA L
LP		O	EE U
AH		2	11 1
31			

GCACTGTCTTGCAGAACCAGAAGAAGGTGGAGTTCAAATAGACATCGTGGTGCTAGCTT 720
661 CGTGACAGAAGCTCTTGGTCTTCTTCCACCTCAAGTTTATCTGTAGCACCACGATCGAA
T V L Q N Q K K V E F K I D I V V L A F -

HS	M M
AT	N N
EU	L L
31	1 1

TCCAGAAGGCCTCCAGCATAGTCTATAAGAAAGAGGGGGAACAGGTGGAGTTCTCCTTCC 780
721 AGGTCTTCCGGAGGTGATCAGATATTCTTTCTCCCCCTTGTCCACCTCAAGAGGAAGG
Q K A S S I V Y K K E G E R V E F S F P -

A
L
U
1

A
L
U
1

M
N
L
1

CACTCGCCTTTACAGTTGAAAAGCTGACGGGAGTGGGAGCTGTGGTGCCAGCGGAGA 840
781 GTGAGCGGAAATGTCAACTTTTCCACTGCCCCTCACCGCTGACACCACCGTCCGCTCT
L A F T V E K L T G S G E L W W Q A E R -

-64-

				P	S		
	H	M	F	M	A		
	P	N	L	N	U		M
	H	L	M	L	3		B
	1	1	1	1	A		0
							2

841 GGGCTTCCTCCTCCAAGTCTTGGATCACCTTTGACCTGAAGAACAAGGAAGTGTCTGTAA

 CCCGAAGGAGGAGGTTTCAAGCTAGTGGAACTGGACTTCTTGTCTCTTACAGACATT 900

 A S S S K S W I T F D L K N K E V S V K -

	B	BS	PS				
	SM	SCADNPAD	A			A	H
	TA	TRVRLUUD	L			L	P
	EE	NFAAAM9E	U			U	H
	23	11224161	1			1	1

AACGGGTTACCCAGGACCCTAAGCTCCAGATGGCAAGAAGCTCCCGCTCCACCTCACCC

 901 TTGCCCAATGGGTCTCTGGGATTCGAGGTCTACCCGTTCTTCGAGGGCGAGGTGGAGTGGG 960

 R V T Q D P K L Q M G K K L P L H L T L -

	BS					BSS	
M	SC HS	D		M	H	SCAHM	
N	TR AT	D		N	P	TRUAN	
L	NF EU	E		L	H	NF9EL	
1	11 31	1		1	1	11631	

TGGCCCAAGGCTTGCCTCAGTATGCTGGCTCTGGAAACCTCACCTTGGCCCTTGAAGCGA

 961 ACGGGGTCCGGAACGGAGTCATACGACCGAGACCTTTGAGTGGGACCGGGAACCTTCGCT 1020

 P Q A L P Q Y A G S G N L T L A L E A K -

		S	BS				
		F	SC			H	D
		A	TR			P	D
		N	NF			H	E
		1	11			1	1

AAACAGGAAAGTTGCATCAGGAAGTGAACCTGGTGGTGATGAGAGCCACTCAGCTCCAGA

 1021 TTTGTCTTTCAACGTAGTCTTCACTTGGACCACCACTACTCTCGGTGAGTGGAGTCT 1080

 T G K L H Q E V N L V V M R A T Q L Q K -

-65-

M		PS	S			
N		ADNPA	DF	AM	DE	A
L		VRLLUJ	DA	LN	DS	L
1		AAAAM9	EN	UL	EP	U
		224418	11	11	11	1
		////	/	/	/	

1081 AAAATTTGACCTGTGAGGTGTGGGGACCCACCTCCCCTAAGCTGATGCTGAGCTTGAAC

 TTTTAAACTGGACACTCCACACCCCTGGGTGGAGGGGATTCCACTACGACTCGAACTTTG 1140

 N L T C E V W G P T S P K L M L S L K L -

M		T		H		DM
N		A		P		DS
L		Q		A		ET
1		1		2		12

1141 TGGAGAACAAGGAGGCAAAGGTCTCGAAGCGGGAGAAGCCGGTGTGGGTGCTGAACCTG

 ACCTCTTGTTCCCTCCGTTTCCAGAGCTTCGCCCTCTTCGGCCACACCCACGACTTGGGAC 1200

 E N K E A K V S K R E K P V W V L N P E -

		H	PS	H
F	D	I	ADPA	I
O	D	NV	VRUJ	N
K	E	FA	AAW9	F
1	1	11	2216	1

1201 AGGCGGGGATGTGGCAGTGTCTGCTGAGTGACTCGGGACAGGTCTGCTGGAATCCAACA

 TCCGCCCCCTACACCGTCACAGACGACTCACTGAGCCCTGTCCAGGACGACCTTAGGTTGT 1260

 A G M W Q C L L S D S G Q V L L E S N I -

S	SA	BHF BS	B
ANA	HNCP	SGNMAANXA	SH
VLU	PCRA	PIUMULHV	PP
AA9	AIFL	1ADLH3AQA	1H
236	2111	21211A421	21

1261 TCAAGGTTCTGCCCACATGGTCCACCCCGGTGCACGCGGATCCCGAGGGTGAGTGTGCC

 AGTTCCAAGACGGGTGTACCAGGTGGGGCCACGTGCCCTAGGGCTCCCACTCACACGGG 1320

 K V L P T W S T P V H A D P E

-66-

MF		BS	S	S	S		
AD		SC	F	DMNA	HNC	A	M
EK		TR	A	RALU	PCR	F	B
11		NF	N	AEA9	AIF	L	G
/		11	1	2346	211	3	2
		/		/	/		

1321 TAGAGTAGCCTGCATCCAGGGACAGGCCCCAGCCGGGTGCTGACACGTCCACCTCCATCT
 ----- 1380
 ATCTCATCGGACGTAGGTCCCTGTCCGGGGTGGCCCCACGACTGTGCAGGTGGAGGTAGA

M	D	M	BS	S		
N	D	N	SC	M	ANA	M
L	E	L	TR	B	VLU	B
1	1	1	NF	D	AA9	0
			11	2	246	2
			/	/	/	

1381 CTTCTCAGTACCTGAACCTCTGGGGGGACCGTCAGTCTTCTTCCCCCAAACCCA
 ----- 1440
 GAAGGAGTCTGGACTTGAGGACCCCTGGCAGTCAGAAGCAGAAGGGGGGTTTTGGGT

A P E L L G G P S V F L F P P K P K -

S		SS		N	
AN	M	HMANAC	DM	M	NS
UL	N	PNVCLUR	DS	A	LP
3A	L	ALAIAGF	ET	E	AM
A3	1	2121461	12	3	31
		/	/	/	/

1441 AGGACACCTCATGATCTCCCGGACCCCTGAGGTACATGCGTGGTGGTGGACGTGAGCC
 ----- 1500
 TCCTGTGGGAGTACTAGAGGGCCTGGGGACTCCAGTGTACGCACCACCACCTGCACTCGG

D T L M I S R T P E V T C V V V D V S H -

M	DM	M		RM	M
N	DS	B		SA	N
L	ET	0		AE	L
1	12	2		12	1
	/			/	

1501 ACGAAGACCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGCAGGTGCATAATGCCA
 ----- 1560
 TGCTTCTGGGACTCCAGTTCAAGTTGACCATGCACCTGCCGCACCTCCACGTATTACGGT

E D P E V K F N W Y V D G V E V H N A K -

	F	FN		S	
M	N	NSS	R	M	R
N	U	UPA	S	A	S
L	4	DBC	A	E	A
1	H	222	1	2	1
	/	/	/	/	/

1561 AGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGGGTGGTCAAGCTCCTCACCG
 ----- 1620
 TCTGTTTCCGGGCCCTCCTCGTCATGTTGTCGTGCATGCCACCAGTCCGAGGAGTGGC

T K P R E E Q Y N S T Y R V V S V L T V -

M	BS		R
N	SC		S
L	TR		A
1	NF		1
	11		

TCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCC
 1621 ----- 1680
 AGGACGTGGTCTCTGACCGACTTACCGTTCCTCATGTTACGTTCCAGAGGTTGTTTCGGG
 L H Q D W L N G K E Y K C K V S N K A L -

N	T		P S	S
L	A		ADNPMMA	A
1	Q		VRLLUNU	U
	1		AAAAML9	9
			2244116	6

TCACAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGTGGGACCCGTGGGGTGCGAG
 1681 ----- 1740
 AGGGTCGGGGGTAGCTCTTTTGGTAGAGGTTTCGGTTTCACCTGGGACCCACGCTC
 P A P I E K T I S K A K

H M	N	HN	S		N	
A N	L	APA	BSAH		S P	R
E L	A	EAE	GFUA		B A	A
3 1	3	321	LI9E		2 1	
			1163			

GGCCACATGGACAGAGGCCGGCTCGGCCACCTCTGCCCTGAGAGTGACCGGTGTACCA
 1741 ----- 1800
 CCGGTGTACCTGTCTCCGGCCGAGCCGGGTGGGAGACGGGACTCTCACTGGCGACATGGT

M	F	A	B	R F	SS
N	N	V	B	S O	AHNNCC
L	U	A	V	A K	VPCCRR
1	4	1	1	1 1	AAIIF
	H				121111

ACCTCTGTCTACAGGGCAGCCCCGAGAACCACAGGTGTACACCTGCCCCCATCCCCGGG
 1801 ----- 1860
 TGGACACAGGATGTCCCGTCGGGGCTCTTGGTGTCCACATGTGGGACGGGGTAGGGCCC
 G Q P R E P Q V Y T L P P S R D -

-68-

S	A	F	BS	BS	B
M	L	O	SC	SC	S
A	U	K	TR	TR	P
1	1	1	NF	NF	M
/	/	/	11	11	1

1861 ATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCCTGGTCAAAGGCTTCTATCCCAGCG 1920

TACTCGACTGGTTCTTGCTCCAGTCGGACTGGACGGACCAGTTTCCGAAGATAGGGTCGC

E L T K N Q V S L T C L V K G F Y P S D -

			F		
			N	H	B
			U	P	B
			4	A	V
			H	2	1

1921 ACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTC 1980

TGTAGCGGCACCTCACCTCTCGTTACCCGTCGGCCTCTTGTTGATGTTCTGGTGGCGAG

I A V E W E S N G Q P E N N Y K T T P P -

	H					B
	M	M	N	H	M	S
	N	B	L	P	N	L
	L	O	A	H	L	U
	1	1	2	1	1	1

1981 CCGTGCCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCACCGTGGACAAGAGCA 2040

GGCAGGACCTGAGGCTGCCGAGGAAGAAGGAGATGTCGTTGAGTGGCACCTGTTCTCGT

V L D S D G S F F L Y S K L T V D K S R -

F			S		
NM	MBX		NF	M	NN
UB	ABM		LA	N	SL
40	EVN		AN	L	IA
H2	211		31	1	13

2041 GGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACT 2100

CCACCGTCGTCCCTTGCAGAAGAGTACGAGGCACTACGTACTCCGACACGTGTTGCTGA

W Q Q G N V F S C S V M H E A L H N H Y -

		S		
M	M	HNC		CXH
B	N	PCR		FMA
D	L	AIF		RAE
2	1	211		133

2101 ACACGCAGAAGAGCCTCTCCCTGTCTCCGGTAAATGAGTGLGACGGCCG 2150

TGTGCGTCTTCTCGGAGAGGGACAGAGGCCCATTTACTCACGCTGCCGGC

T Q K S L S L S P G K .

Example 2: Preparation of the Fusion Proteins from Supernatants of COS Cells

COS cells grown in DME medium supplemented with 10% Calf Serum and gentamicin sulfate at 15 $\mu\text{g/ml}$ were split into DME medium containing 10% NuSerum (Collaborative Research) and gentamicin to give 50% confluence the day before transfection. The next day, CsCl purified plasmid DNA was added to a final concentration of 0.1 to 2.0 $\mu\text{g/ml}$ followed by DEAE Dextran to 400 $\mu\text{g/ml}$ and chloroquine to 100 μM . After 4 hours at 37°C, the medium was aspirated and a 10% solution of dimethyl sulfoxide in phosphate buffered saline was added for 2 minutes, aspirated, and replaced with DME/10% Calf Serum. 8 to 24 hours later, the cells were trypsinized and split 1:2.

For radiolabeling, the medium was aspirated 40 to 48 hours after transfection, the cells washed once with phosphate buffered saline, and DME medium lacking cysteine or methionine was added. 30 minutes later, ^{35}S -labeled cysteine and methionine were added to final concentrations of 30-60 μCi and 100-200 μCi respectively, and the cells allowed to incorporate label for 8 to 24 more hours. The supernatants were recovered and examined by electrophoresis on 7.5% polyacrylamide gels following denaturation and reduction, or on 5% polyacrylamide following denaturation without reduction. The CD4B γ 1 protein gave the same molecular mass with or without reduction, while the CD4E γ 1 and CD4H γ 1 fusion proteins showed molecular masses without reduction of twice the mass observed with reduction, indicating that they formed dimer structures. The CD4 IgM fusion proteins formed large multimers beyond the resolution of the gel system without reduction, and monomers of the expected molecular mass with reduction.

Unlabeled proteins were prepared by allowing the cells to grow for 5 to 10 days post transfection in DME medium containing 5% NuSerum and gentamicin as above. The supernatants were harvested, centrifuged, and purified by batch adsorption to either protein A

trisacryl, protein A agarose, goat anti-human IgG antibody agarose, rabbit anti-human IgM antibody agarose, or monoclonal anti-CD4 antibody agarose. Antibody agarose conjugates were prepared by coupling purified antibodies to cyanogen bromide activated agarose according to the manufacturer's recommendations, and using an antibody concentration of 1 mg/ml. Following batch adsorption by shaking overnight on a rotary table, the beads were harvested by pouring into a sintered glass funnel and washed a few times on the funnel with phosphate buffered saline containing 1% Nonidet P40 detergent. The beads were removed from the funnel and poured into a small disposable plastic column (Quik-Sep QS-Q column, Isolab), washed with at least 20 column volumes of phosphate buffered saline containing 1% Nonidet P40, with 5 volumes of 0.15 M NaCl, 1 mM EDTA (pH 8.0), and eluted by the addition of either 0.1 M acetic acid, 0.1 M acetic acid containing 0.1 M NaCl, or 0.25 M glycine-HCl buffer, pH 2.5.

Example 3: Blockage of Syncytium Formation by the Fusion Proteins

Purified or partially purified fusion proteins were added to HPB-ALL cells infected 12 hours previously with a vaccinia virus recombinant encoding HIV envelope protein. After incubation for 6-8 more hours, the cells were washed with phosphate buffered saline, fixed with formaldehyde, and photographed. All of the full-length CD4 immunoglobulin fusion proteins showed inhibition of syncytium formation at a concentration of 20 µg/ml with the exception of the 4Hyl protein, which was tested only at 5 µg/ml and showed partial inhibition of syncytium formation under the same conditions.

Example 4: Chromium Release Cytolysis Assay

The purified fusion proteins were examined for ability to fix complement in a chromium release assay using vaccinia virus infected cells as a model system. Namalwa (B cell) or HPB-ALL (T cell) lines

were infected with vaccinia virus encoding HIV envelope protein, and 18 hours later were radiolabeled by incubation in 1 mCi/ml sodium ^{51}Cr chromate in phosphate buffered saline for 1 hour at 37°. The labeled cells were centrifuged to remove the unincorporated chromate, and incubated in microtiter wells with serial dilutions of the CD4 immunoglobulin fusion proteins and rabbit complement at a final concentration of 40%. After 1 hour at 37°, the cells were mixed well, centrifuged, and the supernatants counted in a gamma-ray counter. No specific release could be convincingly documented.

Example 5: Binding of the CD4Eyl Protein to Fc Receptors

Purified CD4Eyl fusion protein was tested for its ability to displace radiolabeled human IgG1 from human Fc receptors expressed on COS cells in culture. The IgG1 was radiolabeled with sodium ^{125}I iodide using 1 mCi of iodide, 100 μg of IgG1, and two idobeads (Pierce). The labeled protein was separated from unincorporated counts by passage over a Sephadex G25 column equilibrated with phosphate buffered saline containing 0.5 mM EDTA and 5% nonfat milk. Serial dilutions of the CD4Eyl fusion protein or unlabeled IgG1 were prepared and mixed with a constant amount of radiolabeled IgG1 tracer. After incubation with COS cells bearing the FcRI and RcrII receptors at 4°C for at least 45 minutes in a volume of 20 μl , 200 μl of a 3:2 mixture of dibutyl to dioctyl phthalates were added, and the cells separated from the unbound label by centrifugation in a microcentrifuge for 15 to 30 seconds. The tubes were cut with scissors, and the cell pellets counted in a gamma-ray counter. The affinity of the CD4Eyl protein for receptors was measured in parallel with the affinity of the authentic IgG1 protein, and was found to be the same, within experimental error.

Example 6: Stable Expression of the Fusion Construct pCD4Eyl in Baby Hamster Kidney Cells

-72-

Twenty-four hours before transfection, 0.5×10^6 baby hamster kidney cells (BHK; ATCC CCL10) were seeded in a 25 cm^2 culture flask in Dulbecco's modified Eagle's medium (DMEM) containing 10% of fetal calf serum (FCS). The cells were cotransfected with a mixture of the plasmids pCD4Eyl (20 μg), pSV2dhfr (5 μg ; Lee *et al.*, *Nature* 294:228-232 (1981)) and pRMH140 (5 μg , Hudziak *et al.*, *Cell* 31:137-146 (1982)) according to a modified calcium phosphate transfection technique as described in Zettlmeissl *et al.* (*Behring Inst. Res. Comm.* 82:26-34 (1988)). 72 h post-transfection, cells were split 1:3 to 1:4 (60 mm culture dishes) and resistant colonies were selected in DMEM medium containing 10% FCS, 400 $\mu\text{g}/\text{ml}$ G418 (Geneticin, Gibco) and 1 μM methotrexate (selection medium). The medium was changed twice a week. The resistant colonies (40-100/transfection) appeared 10-15 day post-transfection and were further propagated either as a mixture of clones (i.e., BHK-MK1) or as individually isolated clones. For the determination of the relative expression levels, clone mixtures or individual clones were grown to confluency in T25 culture flasks, washed twice with protein-free DMEM medium, and incubated for 24 h with 5 ml protein-free DMEM medium. These media were collected and subjected to a human IgG specific ELISA in order to determine the relative expression levels of the CD4-IgG1 fusion protein CD4Eyl. For further analysis an individual clone (BHK-UC3) was chosen due to its high relative expression levels.

Example 7: Detection of the CD4Eyl Protein in Culture Supernatants

For ^{35}S methionine labeling of cells, the clone BHK-UC3 and untransfected BHK cells (control) were grown to confluency in T25 culture flasks and subsequently incubated for two hours in HamF12 medium without methionine. Labeling was achieved by incubating 24 h in 2.5 ml of the same medium containing 100 μCi ^{35}S methionine (1070 Ci/mole, Amersham). For the preparation of cell lysates, the labeled

-73-

cells were harvested in 1 ml of phosphate buffered saline, pH 7.2 (PBS) and lysed by repetitive freezing and thawing. Cleared lysates (after centrifugation 20000 rpm, 20 min) and culture supernatants were incubated with Protein A-Sepharose (Pharmacia) and the bound material was analyzed on a 10% SDS-Protein A-Sepharose (Pharmacia) and the bound material was analyzed on a 10% SDS-gel according to Laemmli (*Nature* 227:680-685 (1970)), which was subsequently autoradiographed. A specific band of about 80 KDa can be detected only in the supernatant of clone BHK-UC3, which is absent in the lysate of clone BHK-UC3 and in the respective controls.

Example 8: Purification of the Protein CD4Eyl from Culture Supernatants

In order to demonstrate that the fusion protein coded by the plasmid pCD4Eyl can be obtained in high quantities, the clone BHK-UC3 was grown in 1750 cm² roller bottles in selection medium (500 ml). Confluent monolayers were washed twice with protein-free DMEM medium (200 ml) and further incubated for 48 h with protein-free DMEM medium (500 ml). The conditioned culture supernatants (1-2 l) and respective supernatants from untransfected BHK cells were cleared by centrifugation (9000 rpm, 30 min) and microfiltered through a 0.45 µm membrane (Nalgene). After addition of 1% (v/v) of 1.9 M Tris-HCl buffer, pH 8.6, the conditioned medium was absorbed to a Protein A-Sepharose column equilibrated with 50 mM Tris-HCl pH 8.6 buffer containing 150 mM NaCl (4°C). The loaded column was washed with 10 column volumes of equilibration buffer. Elution of the CD4-IgG1 fusion protein CD4Eyl was achieved with 0.1 M sodium citrate buffer, pH 3, followed by immediate neutralization of the column efflux to pH 8 by Tris-base. The peak fractions were pooled, and the pool was analyzed on a Coomassie blue stained SDS-gel resulting in a band of the expected size (80 KDa), and which reacted with a polyclonal anti-human IgG heavy chain antibody and a mouse monoclonal anti-CD4

antibody (BMA040, Behringwerke) in Western Blots. The yields of purified fusion proteins obtained by the given procedure is 5-18 mg/24 h/1 culture supernatant. The respective value for a BHK clone mixture (about 80 resistant clones; BHK-MK1) as described above was 2-3 mg/24 h/1.

Example 9: Physical and Biological Characterization of the CD4Eyl Fusion Protein

As proven by SDS-electrophoresis on 10-15% gradient gels (Phast-System, Pharmacia) under non-reductive conditions, the CD4Eyl fusion protein migrates at the position of a homodimer (about 160 KDa) like a non-reduced mouse monoclonal antibody. This result is supported by analytical equilibrium ultracentrifugation, where the fusion protein behaves as a homogeneous dimeric molecule of about 150 KDa. The absorbance coefficient of the protein was determined as $A_{280} = 18 \text{ cm}^2/\text{mg}$ using the quantitative protein determination according to Bradford (Anal. Biochem. 72:248-254 (1976)).

The CD4Eyl-fusion protein shows specific complex formation with a solubilized β gal-gp120 fusion protein (pMB1790; Broker *et al.*, Behring Inst. Res. Commun. 82:338-348 (1988)) expressed in *E. coli*. In this protein (110 KDa), a major part of the HIV gp120 protein (Val49-Trp646) is fused to β -galactosidase (amino acids 1-375). In a control experiment a 67-KDa β gal-HIV 3'orf fusion protein (β gal1-375; 3'orf Prol4-Asp123) showed no complex formation. In these experiments, the CD4Eyl-protein was incubated with the respective fusion protein in molar ratios of about 5:1. The complex was isolated by binding to Protein A-Sepharose and the Protein A-Sepharose bound proteins--together with relevant controls--were analyzed on 10-15% gradient SDS-gels (Phast-System, Pharmacia).

The CD4Eyl fusion protein binds to the surface of HIV (HIV1/HTLV-IIIB) infected cultured T4-lymphocytes as determined by direct immunofluorescence with fluorescein-isothiocyanate (FITC) labeled

-75-

CD4Eyl protein. It blocks syncytia formation in cultured T4-lymphocytes upon HIV infection (0.25 TCID₅₀/cell) at a concentration of 10 µg/ml. Furthermore, HIV-infected cultured T4-lymphocytes (subclone of cell line H9) are selectively killed upon incubation with CD4Eyl in the presence or absence of complement: To a highly (>50%) HIV infected culture of T4-lymphocytes (10⁶ cells/ml) 50, 10 or 1 µg/ml CD4Eyl fusion protein was added in the presence or absence of guinea pig complement. Cells were observed for specific killing by the fusion protein, which is defined by the percentage of killed cells after 3 days in relation to viable cells in the culture at the beginning of the experiment corrected by the values for unspecific killing observed in control cultures, lacking the CD4Eyl fusion protein (Table 5, Experiment I). Surprisingly, addition of CD4Eyl protein to the infected T4 cells in the absence of complement resulted in similar specific killing rates as in the presence of complement (Table 5, Experiment II). This result demonstrates a complement independent cytolytic effect of CD4Eyl on HIV infected T-lymphocytes in culture.

Table 5

<u>No. Experiment</u>	<u>Assay System</u>	<u>Specific Killing (%)</u>
I	non-infected T4-cells + 50 µg/ml CD4Eyl + Compl.	0.7
	infected T4-cells + 50 µg/ml CD4Eyl + Compl.	35.1
	infected T4-cells + 10 µg/ml CD4Eyl + Compl.	25.1
	infected T4-cells + 1 µg/ml CD4Eyl + Compl.	25
II	infected T4-cells + 10 µg/ml CD4Eyl + Compl.	49.9
	infected T4-cells + 10 µg/ml CD4Eyl + Compl.	69.4

Having now fully described this invention, it will be appreciated by those skilled in the art that the same can be performed with any wide range of equivalent parameters of composition, conditions, and methods of preparing such fusion proteins without departing from the spirit or scope of the invention or any embodiment thereof.

□

□

1

3

International Application No: PCT/

/

MICROORGANISMS

Continued Sheet > connection with the microorganism referred to in: page 23, line 20 of the description *

A. IDENTIFICATION OF DEPOSIT *

Further deposits are identified on an additional sheet ☒ *

Name of depository institution *

AMERICAN TYPE CULTURE COLLECTION

Address of depository institution (including postal code and country) *

12301 Parklawn Drive
Rockville, Maryland 20852

United States of America

Date of deposit *

20 January 1988

Accession Number *

67611

B. ADDITIONAL INDICATIONS * (Leave blank if not applicable). This information is contained on a separate attached sheet ☐Escherichia coli bearing plasmids: MC1061/p3 (pCD4Hgamml)

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE * (If the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS * (Leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later * (Specify the general nature of the indications e.g., "Accession Number of Deposit")

E. ☐ This sheet was received with the international application when filed (to be checked by the Receiving Office)

(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau is:

(Authorized Officer)

(January 1985)

International Application No: PCT/

1

MICROORGANISMS

Optional Sheet 2: connection with the microorganism referred to on page 23, line 25 of the description *

A. IDENTIFICATION OF DEPOSIT *

Further deposits are identified on an additional sheet ☒ *

Name of depository institution *

AMERICAN TYPE CULTURE COLLECTION

Address of depository institution (including postal code and country) *

12301 Parklawn Drive
Rockville, Maryland 20852
United States of America

Date of deposit *

20 January 1988

Accession Number *

67610

B. ADDITIONAL INDICATIONS * (Leave blank if not applicable). This information is continued on a separate attached sheet ☐Escherichia coli bearing plasmids: MC1061/p3 (pCD4Egammal)

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE * (If the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS * (Leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later * (Specify the general nature of the indications e.g., "Accession Number of Deposit")

E. ☐ This sheet was received with the international application when filed (to be checked by the receiving Office)

(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau is:

(Authorized Officer)

(January 1985)

International Application No: PCT/

1

MICROORGANISMS	
Optional Sheet in connection with the microorganism referred to on page <u>24</u> , line <u>6</u> of the description *	
A. IDENTIFICATION OF DEPOSIT *	
Further deposits are identified on an additional sheet <input checked="" type="checkbox"/> *	
Name of depository institution *	
AMERICAN TYPE CULTURE COLLECTION	
Address of depository institution (including postal code and country) *	
12301 Parklawn Drive Rockville, Maryland 20852 United States of America	
Date of deposit *	Accession Number *
20 January 1988	67608
B. ADDITIONAL INDICATIONS * (leave blank if not applicable. This information is continued on a separate attached sheet <input type="checkbox"/>)	
<u>Escherichia coli</u> bearing plasmids: MC1061/p3 (pCD4Pmu)	
C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE * (if the indications are not for all designated States)	
D. SEPARATE FURNISHING OF INDICATIONS * (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later * (Specify the general nature of the indications e.g., "Accession Number of Deposit")	
E. <input type="checkbox"/> This sheet was received with the international application when filed (to be checked by the receiving Office)	
(Authorized Officer) <i>Ante Hart</i>	
<input type="checkbox"/> The date of receipt (from the applicant) by the International Bureau **	
000	
(Authorized Officer)	

(January 1985)

International Application No: PCT/

/

MICROORGANISMSOptional Sheet in connection with the microorganism referred to on page 24, line 1 of the description.**A. IDENTIFICATION OF DEPOSIT :**Further deposits are identified on an additional sheet ☐.

Name of Depository Institution :

AMERICAN TYPE CULTURE COLLECTION

Address of depository institution (including postal code and country) :

12301 Parklawn Drive
Rockville, Maryland 20852
~~United States of America~~

Date of deposit :

20 January 1988

Accession Number :

67609

B. ADDITIONAL INDICATIONS : (Leave blank if not applicable). This information is continued on a separate attached sheet ☐.Escherichka coli bearing plasmids: MC1061/p3 (pCD4Mmu)**C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE :** (If the indications are not for all designated States)**D. SEPARATE FURNISHING OF INDICATIONS :** (Leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later. (Specify the general nature of the indications e.g., "Accession Number of Deposit.")

E. ☐ This sheet was received with the international application when filed (to be checked by the receiving Office)

(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau is :

was

(Authorized Officer)

(January 1988)

WHAT IS CLAIMED IS:

1. A fusion protein gene comprising 1) the DNA sequence of CD4, or fragment thereof which binds to HIV gp120, and 2) the DNA sequence of an immunoglobulin heavy chain, wherein the DNA sequence which encodes the variable region of said immunoglobulin chain has been replaced with the DNA sequence which encodes CD4, or said gp120 binding fragment thereof.

2. The fusion protein gene of claim 1, wherein the DNA sequence which encodes said fragment of CD4 comprises the following DNA sequence:

```
CAATGAACCGGG
+----- 120
GTTACTTGGCCC

121 GAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTCCTCCCAGCAGCCACTC
-----+-----+-----+-----+-----+----- 180
CTCAGGGAAAATCCGTGAACGAAGACCACGACGTTGACCGCGAGGAGGGTCGTCGGTGAG

181 AGGGAAAGAAAGTGGTGCTGGGCAAAAAGGGGATACAGTGGAACTGACCTGTACAGCTT
-----+-----+-----+-----+-----+----- 240
TCCCTTTCTTTCACCACGACCCGTTTTTCCCTATGTCACCTTGACTGGACATGTCGAA

241 CCCAGAAGAAGAGCATACAATTCCACTGGAAAACTCCAACCAGATAAAGATTCTGGGAA
-----+-----+-----+-----+-----+----- 300
GGGTCTTCTTCTCGTATGTTAAGGTGACCTTTTTGAGGTTGGTCTATTTCTAAGACCCTT

301 ATCAGGGCTCCTTCTTAATAAGGTCCATCCAAGCTGAATGATCGCGCTGACTCAAGAA
-----+-----+-----+-----+-----+----- 360
TAGTCCCAGGAAGAATGATTTCCAGGTAGGTTGACTTACTAGCGCGACTGAGTTCTT

361 GAAGCCTTTGGGACCAAGGAACTTCCCCCTGATCATCAAGAATCTTAAGATAGAAGACT
-----+-----+-----+-----+-----+----- 420
CTTCGGAACCCGTGTTCTTTGAAGGGGGACTAGTAGTTCTTAGAATTCTATCTTCTGA
```

3. The fusion protein gene of claim 1, wherein said DNA sequence which encodes said fragment of CD4 comprises the following DNA sequence:

CAATGAACCCGGG
-+----- 120
GTTACTTGGCCC

121
GAGTCCCTTTTAGGCACTTGCTTCTGGTGTGCAACTGGCGCTCTCTCCAGCAGCCACTC
-----+-----+-----+-----+-----+----- 180
CTCAGGGAAAATCCGTGAACGAAGACCACGACGTTGACCGCGAGGAGGGTCGTCGGTGAG

181
AGGGAAAGAAAGTGGTGTGGGCAAAAAGGGGATACAGTGGAACTGACCTGTACAGCTT
-----+-----+-----+-----+-----+----- 240
TCCCTTTCTTTCACCACGACCCGTTTTTCCCTATGTCACTTGACTGGACATGTCGAA

241
CCCAGAAGAAGAGCATACAATTCCACTGGAAAACTCCAACCAGATAAAGATTCTGGGAA
-----+-----+-----+-----+-----+----- 300
GGGTCTTCTTCTCGTATGTTAAGGTGACCTTTTGGAGTTGGTCTATTCTAAGACCCTT

301
ATCAGGGCTCCTTCTTAATAAAGGTCCATCCAAGCTGAATGATCGCGCTGACTCAAGAA
-----+-----+-----+-----+-----+----- 360
TAGTCCCAGGAAGAATTGATTCCAGGTAGGTTGACTTACTAGCGCGACTGAGTTCTT

361
GAAGCCTTTGGGACCAAGGAAACTTCCCCCTGATCATCAAGAATCTTAAGATAGAAGACT
-----+-----+-----+-----+-----+----- 420
CTTCGGAAACCTGGTTCTTTGAAGGGGGACTAGTAGTTCTTAGAATTCTATCTTCTGA

421
CAGATACTTACATCTGTGAAGTGGAGGACCAGAAGGAGGAGGTGCAATTGCTAGTGTTCG
-----+-----+-----+-----+-----+----- 480
GTCTATGAATGTAGACACTTCACCTCCTGGTCTTCTCCTCCACGTTAACGATCACAAGC

481
GATTGACTGCCAACTCTGACACCCACCTGCTTCAGGGGCAGAGCCTGACCCTGACCTTGG
-----+-----+-----+-----+-----+----- 540
CTAACTGACGGTTGAGACTGTGGGTGGACGAAGTCCCCGTCTCGGACTGGGACTGGAACC

541 AGAGCCCCCTGGTAGTAGCCCTCAGTGCATGTAGGAGTCCAAGGGGTAAAAACATAC
-----+-----+-----+-----+----- 600
TCTCGGGGGGACCATCATCGGGGAGTCACGTTACATCCTCAGGTTCCCATTTTTGTATG
601 AGGGGGGGAAGACCCCTCTCCGTGTCTCAG
-----+-----+-----+-----+-----
TCCCCCCTTCTGGGAGAGGCACAGAGTC

or a degenerate variant thereof.

4. The fusion protein gene of claim 1, wherein said immunoglobulin chain is of the class IgM, IgG1 or IgG3.

5. A fusion protein gene comprising 1) the DNA sequence of CD4, or fragment thereof which binds to HIV gp120, and 2) the DNA sequence of an immunoglobulin light chain, wherein the DNA sequence which encodes the variable region of said immunoglobulin light chain has been replaced with the DNA sequence which encodes CD4, or HIV gp120-binding fragment thereof.

6. The fusion protein gene of claim 5, wherein the DNA sequence which encodes said fragment of CD4 comprises the following DNA sequence:

CAATGAACCGGG
-----+----- 120
GTTACTTGGCCC
121 GAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTGGCGCTCCTCCCAGCAGCCACTC
-----+-----+-----+-----+----- 180
CTCAGGGAAAATCCGTGAACGAAGACCACGACGTTGACCGCGAGGAGGGTCGTCGGTGAG
181 AGGGAAAGAAAGTGGTGCTGGGCAAAAAAGGGGATACAGTGGAAGTACCTGTACAGCTT
-----+-----+-----+-----+----- 240
TCCCTTTCTTTCACCACGACCCGTTTTTCCCTATGTCACCTTGACTGGACATGTCGAA

241 CCCAGAAGAAGAGCATACAATTCCACTGGAAAACTCCAACCAGATAAAGATTCTGGGAA 300
-----+-----+-----+-----+-----+-----
GGGTCTTCTTCTCGTATGTTAAGGTGACCTTTTTGAGGTTGGTCTATTCTAAGACCCCTT

301 ATCAGGGCTCCTTCTTAATAAGGTCCATCCAAGCTGAATGATCGCGCTGACTCAAGAA 360
-----+-----+-----+-----+-----+-----
TAGTCCCAGGAAGAATTGATTTCCAGGTAGGTTGACTTACTAGCGCGACTGAGTTCTT

361 GAAGCCTTTGGGACCAAGGAACTTCCCCCTGATCATCAAGAATCTTAAGATAGAAGACT 420
-----+-----+-----+-----+-----+-----
CTTCGGAAACCCTGGTTCCTTTGAAGGGGGACTAGTAGTTCTTAGAATTCTATCTTCTGA

421 CAGATACTTACATCTGTGAAGTGGAGGACCAGAAGGAGGAGGTGCAATTGCTAGTGTTCTG 480
-----+-----+-----+-----+-----+-----
GTCTATGAATGTAGACACTTCACCTCCTGGTCTTCCTCCTCCAGTTAACGATCACAAGC

481 GATTGACTGCCAACTCTGACACCCACCTGCTTC
-----+-----+-----+-----
CTAACTGACGGTTGAGACTGTGGGTGGACGAAG

or a degenerate variant thereof.

7. The fusion protein gene of claim 5, wherein the DNA sequence which encodes said fragment of CD4 comprises the following DNA sequence:

CAATGAACCGGG 120
-----+-----
GTTACTTGGCCC

121 GAGTCCCTTTTAGGCACTTGCTTCTGGTGCTGCAACTG6CGCTCCTCCAGCAGCCACTC 180
-----+-----+-----+-----+-----+-----
CTCAGGGAAAATCCGTGAACGAAGACCACGACGTTGACCGCGAGGAGGGTCGTCGGTGAG

ATCC under the terms of this Budapest Treaty under Accession No. 67609.

11. The vector of claim 8, having the identifying characteristics of pCD4P μ , which has been deposited in E. coli at the ATCC under the Budapest Treaty under Accession No. 67608.

12. The vector of claim 8, having the identifying characteristics of pCD4E γ 1, which has been deposited in E. coli at the ATCC under the terms of the Budapest Treaty under Accession No. 67610.

13. A vector comprising the fusion protein gene of claim 5.

14. A host transformed with the vector of claim 8.

15. The host of claim 14 which expresses an immunoglobulin light chain together with the expression product of said fusion protein gene to give an immunoglobulin-like molecule which binds to gp120.

16. A host transformed with the vector of claim 13.

17. The host of claim 16 which expresses an immunoglobulin heavy chain together with the expression product of said fusion protein gene to give an immunoglobulin-like molecule which binds to HIV or SIV gp120.

18. The host of claim 17, wherein said immunoglobulin heavy chain is of the immunoglobulin class IgM, IgG1 or IgG3.

19. A method of producing a fusion protein comprising CD4, or fragment thereof which binds to gp120, and immunoglobulin heavy chain, wherein the variable region of the immunoglobulin chain has been

substituted with CD4, or fragment thereof which binds to HIV or SIV gp120, which comprises

cultivating in a nutrient medium under protein-producing conditions, a host strain transformed with the vector of claim 6, said vector further comprising expression signals which are recognized by said host strain and direct expression of said fusion protein, and

recovering the fusion protein so produced.

20. The method of claim 19, wherein said host strain is a myeloma cell line which produces immunoglobulin light chains and said fusion protein comprises an immunoglobulin heavy chain of the class IgM, IgG1 or IgG3, wherein an immunoglobulin-like molecule comprising said fusion protein is produced.

21. A method of producing a fusion protein comprising CD4, or fragment thereof which binds to gp120, and an immunoglobulin light chain, wherein the variable region of the immunoglobulin chain has been substituted with CD4, or fragment thereof which binds to HIV or SIV gp120, which comprises:

cultivating in a nutrient medium under protein-producing conditions, a host strain transformed with the vector of claim 8, said vector further comprising expression signals which are recognized by said host strain and direct expression of said fusion protein, and

recovering the fusion protein so produced.

22. The method of claim 21, wherein said host produces immunoglobulin heavy chains of the class IgM, IgG1 and IgG3 together with said fusion protein to give an immunoglobulin-like molecule which binds to HIV-gp120.

23. A fusion protein comprising CD4, or fragment thereof which is capable of binding to HIV or SIV gp120, fused at the C-terminus to a second protein which comprises an immunoglobulin heavy chain of the class IgM, IgG1 or IgG3, wherein the variable region of said heavy chain immunoglobulin has been replaced with CD4, or HIV gp120-binding fragment thereof.

24. The fusion protein CD4H γ 1.

25. The fusion protein CD4M μ .

26. The fusion protein CD4P μ .

27. The fusion protein CD4E γ 1.

28. The fusion protein CD4B γ 1.

29. The fusion protein of claim 23 which is detectably labeled.

30. The fusion protein of claim 23, further comprising a therapeutic agent, radiolabel or NMR imaging agent linked to said fusion protein.

31. A immunoglobulin-like molecule, comprising the fusion protein of claim 23 and an immunoglobulin light chain.

32. The immunoglobulin-like molecule of claim 31, further comprising a detectable label.

33. The immunoglobulin-like molecule of claim 31, further comprising a therapeutic agent, radiolabel or NMR imaging agent linked to said immunoglobulin-like molecule.

34. A fusion protein comprising CD4, or fragment thereof which binds to HIV gp120, fused at the C-terminus to a second protein comprising an immunoglobulin light chain where the variable region has been deleted.

35. The fusion protein of claim 23, wherein said CD4 fragment comprises the following amino acid sequence:

M N R G
V P F R H L L L V L Q L A L L P A A T Q
G K K V V L G K K G D T V E L T C T A S
Q K K S I Q F H W K N S N Q I K I L G N
Q G S F L T K G P S K L N D R A D S R R
S L W D Q G N F P L I I K N L K I E D S
D T Y I C E V E D Q K E E V Q L L V F G
L T A N S D T H L L Q

36. The fusion protein of claim 23, wherein said CD4 fragment comprises the following amino acid sequence:

M N R G
V P F R H L L L V L Q L A L L P A A T Q
G K K V V L G K K G D T V E L T C T A S
Q K K S I Q F H W K N S N Q I K I L G N
Q G S F L T K G P S K L N D R A D S R R
S L W D Q G N F P L I I K N L K I E D S
D T Y I C E V E D Q K E E V Q L L V F G
L T A N S D T H L L Q G Q S L T L T L E
S P P G S S P S V Q C R S P R G K N I Q
G G K T L S V S Q

37. The fusion protein of claim 34 which is detectably labeled.

38. The fusion protein of claim 34, further comprising a

therapeutic agent, radiolabel or NMR imaging agent linked to said fusion protein.

39. An immunoglobulin-like molecule comprising the fusion protein of claim 34 and an immunoglobulin heavy chain of the class IgM, IgG1 or IgG3.

40. The immunoglobulin-like molecule of claim 39, further comprising a detectable label.

41. The immunoglobulin-like molecule of claim 39, further comprising a therapeutic agent, radiolabel or NMR imaging agent linked to said immunoglobulin-like molecule.

42. A complex comprising the fusion protein of claim 23 and HIV or SIV gp120.

43. The complex of claim 42, wherein said gp120 is a part of an HIV or SIV, is expressed on the surface of an HIV or SIV-infected cell or is present in solution.

44. A complex comprising the fusion protein of claim 34 and HIV or SIV gp120.

45. The complex of claim 43, wherein said gp120 is a part of an HIV or SIV, is expressed on the surface of an HIV or SIV infected cell or is present in solution.

46. A method of treating HIV or SIV infections, comprising administering the fusion protein of claim 23 to an animal.

47. The method of claim 46, wherein said animal is a human.

-87-

48. A method of treating HIV or SIV infections, comprising administering the fusion protein of claim 34 to an animal.

49. The method of claim 48, wherein said animal is a human.

50. A method for the detection of HIV or SIV gp120 in a sample, comprising

- (a) contacting a sample suspected of containing HIV or SIV gp120 with the fusion protein of claim 23, and
- (b) detecting whether a complex is formed.

51. The method of claim 50, wherein said fusion protein is detectably labeled.

52. A method for the detection of HIV or SIV gp120 in a sample, comprising

- (a) contacting a sample suspected of containing HIV or SIV gp120 with the fusion protein of claim 34, and
- (b) detecting whether a complex has formed.

53. The method of claim 52, wherein said fusion protein is detectably labeled.

INTERNATIONAL SEARCH REPORT

International Application

PCT/US89/00238

CLASSIFICATION OF SUBJECT MATTER (1) According to International Classification (IPC) or to other National Classification or the IPC
 According to International Classification (IPC) or to other National Classification or the IPC
 IPC(4): C 12 N 15/00, C 12 P 21/02; A 61 K 39/395, 49/00;
 US Cl.: 530/324

II. FIELDS SEARCHED

Minimum Documentation Searched

Classification System

Classification Symbols

U.S.

435/60, 172.3; 514/12; 530/324, 387, 388; 535/5; 536/27

Documentation Searched other than Minimum Documentation
 to the extent that such documents are included in the fields searched

Databases: Chemical Abstracts Services Online (File CA, 1967-1989; File Biosis, 1969-1989). Automated Patent System (US PAT 1975-1989).

III. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No. 1
E, Y	US, A, 4,816,567, S. Cabilly et al. Published 28 March 1989.	1-53
Y	EP 0 125 023 A1, S. Cabilly et al. Published 14 November 1984.	1-53
Y	Nature, Volume 312, Published 13 December 1984, G.L. Boulianne et al., "Production of functional chimaeric mouse/human antibody", see pages 643-646.	1-53
Y	Proceedings of the National Academy of Sciences, U.S.A., Volume 84, Published May 1987, N.R.J. Gascoigne et al., "Secretion of a chimeric T-cell receptor-immunoglobulin protein", see pages 2936-2940.	1-53
Y	Proceedings of the National Academy of Sciences, U.S.A., Volume 81, Published November, 1984, S.L. Morrison et al., "Chimeric human antibody molecules: Mouse antigen-binding domains with human constant regions domains", see pages 6851-6855.	1-53

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another claim or other special reason (as specified)
- "O" document relating to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

- "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is considered with one or more other such documents, such combination being obvious to a person skilled in the art.

- "Z" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

18 May 1989

Date of Making of the International Search Report

12 June 1989

International Searching Authority

ISA/US

Signature of Authorized Officer

Denise C. Bernstein

IN DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category: Citation of Document with indication where appropriate, of the relevant passages Relevant to Claim No.

- | | | |
|------|--|------|
| Y | Proceedings of the National Academy of Sciences, U.S.A., Volume 84, Published April, 1987, T.J. Palker et al., "A conserved region at the COOH terminus of human immunodeficiency virus gp120 envelope protein contains an immunodominant epitope", see pages 2479-2483. | 1-53 |
| Y | Nature, Volume 312, Published 13 December 1984 M.S. Neuberger et al., "Recombinant antibodies possessing novel effector functions", see pages 604-608. | 1-53 |
| T | Nature, Volume 337, Published 9 February 1989, D.J. Capon et al., "Designing CD4 immunoadhesins for AIDS therapy:", see pages 525-531. | 1-53 |
| P, Y | Proceedings of the National Academy of Sciences, U.S.A., Volume 85, Published May, 1988, J.R. Rusche et al., "Antibodies that inhibit fusion of human immunodeficiency virus infected cells bind a 24-amino acid sequence of the viral envelope, gp120, see pages 3198-3202. | 1-53 |